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1 **How do backward walking ants (*Cataglyphis velox*) cope with navigational**  
2 **uncertainty?**

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21

22 **ABSTRACT**

23 Current opinion in insect navigation assumes that animals need to align with the goal  
24 direction to recognise familiar views and approach it. Yet, ants sometimes drag  
25 heavy food items backward to the nest and it is still unclear to what extent they rely  
26 on visual memories while doing so. In this study displacement experiments and  
27 alterations of the visual scenery reveal that ants do indeed recognise and use the  
28 learnt visual scenery to guide their path towards the nest while walking backward. In  
29 addition, the results show that backward ants estimate their directional uncertainty by  
30 integrating multiple cues such as visual familiarity, the state of their path integrator  
31 and the time spent backward. A simple mechanical model based on repulsive and  
32 attractive visual memories captures the results and explains how visual navigation  
33 can be performed backwards.

34

35 **Keywords:** ants, backward movement, navigation, peeking, route following,  
36 uncertainty, view-based navigation

37

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39

## 40 INTRODUCTION

41

42 Central place foragers – such as desert ants – exhibit formidable navigational  
43 skills to find food and their way back home during numerous daily foraging trips  
44 (Collett, Graham, & Durier, 2003; Heinze, Narendra, & Cheung, 2018; Wehner,  
45 2003). These ground dwellers rely on a set of navigational strategies such as path  
46 integration (Wehner & Srinivasan, 2003; Wittlinger, Wehner, & Wolf, 2006) and visual  
47 scene navigation (Cheng, Narendra, Sommer, & Wehner, 2009; Zeil, 2012). Path  
48 integration (PI) is the capacity to return to a starting point by constantly updating the  
49 directional and distance information (based on celestial compass cues and a stride  
50 odometer) during an outbound trip whereas visual scene navigation provides  
51 directional information by comparing memorised and currently perceived views of  
52 terrestrial cues. The literature agrees that ants continuously integrate the directional  
53 dictates of these different strategies, rather than switching between them (Collett,  
54 2012; Hoinville & Wehner, 2018; Legge, Wystrach, Spetch, & Cheng, 2014; Reid,  
55 Narendra, Hemmi, & Zeil, 2011; Wystrach, Mangan, & Webb, 2015).

56 Current models of insect visual navigation capture well the behaviour of  
57 forward navigating ants (Baddeley, Graham, Husbands, & Philippides, 2012;  
58 Hoinville & Wehner, 2018; Wystrach, Beugnon, & Cheng, 2011; Wystrach, Cheng,  
59 Sosa, & Beugnon, 2011; Zeil, 2012) however, how ants navigate while dragging a  
60 heavy food item backward remains unclear (Ardin, Mangan, & Webb, 2016; Pfeffer &  
61 Wittlinger, 2016; Schwarz, Mangan, Zeil, Webb, & Wystrach, 2017). Despite their  
62 irregular backward foot strides the ants' PI system seems as accurate as during  
63 forward movement (Pfeffer, Wahl, & Wittlinger, 2016; Pfeffer & Wittlinger, 2016),  
64 however, guidance based on terrestrial visual cues seems disrupted (Schwarz et al.,  
65 2017). Indeed, evidence suggests that to recognise the familiar terrestrial scenery

66 ants need to align their body in the familiar forward direction (Narendra, Gourmaud, &  
67 Zeil, 2013; Wystrach, Cheng, et al., 2011; Zeil, 2012). This is probably why ants  
68 dragging a food item backward occasionally display a so-called 'peeking' behaviour:  
69 the ant stops pulling, drops its food item and turns around to look forward. If the  
70 scenery is familiar, the ant quickly returns to her food item and adjusts her backward  
71 path in the newly corrected homing direction. It seems clear that during these few  
72 moments facing forward in a familiar direction, ants recover and store the correct  
73 direction; and subsequently rely on celestial cues to maintain this new bearing when  
74 traveling backward (Schwarz et al., 2017). In this case navigation is discretised into  
75 different sources of information being used sequentially rather than simultaneously.  
76 Also, 'peeking' involves the decision to trigger a distinct and observable behaviour  
77 when navigational information is needed. This behaviour therefore provides a good  
78 opportunity to investigate how ants estimate their navigational uncertainty and as a  
79 corollary, which navigational information they have access to.

80 Here two experiments with backward walking ants were carried out to  
81 investigate the following questions: (1) Do ants still pay attention to visual terrestrial  
82 cues when walking backward? (2) Does this visual information enable ants to guide  
83 their backward path? (3) Which information is used by ants to estimate uncertainty  
84 and trigger a peeking behaviour?

85

## 86 **METHODS**

87

### 88 **Study animal and site**

89

90 The experiments were carried out with Spanish desert ants *Cataglyphis velox*  
91 on a field site with diverse grass and bush vegetation at the outskirts of Seville

92 (37°19'55"N 5°59'20"W) during June 2017 and 18. *Cataglyphis velox* show typical  
93 characteristics of a desert ant such as diurnality, thermophily and solitary foraging  
94 (Cerde, 2001). As in other ant species, navigation and orientation in *C. velox* is  
95 predominantly based on vision derived from terrestrial and celestial cues (Mangan &  
96 Webb, 2012; Wystrach et al., 2015).

97

## 98 **General methods**

99 Two experiments were conducted: Experiment 1 in 2017 and Experiment 2 in  
100 2018. Both set-ups shared the following methods. Ants were restricted to forage on a  
101 straight route (for specific dimensions see below) between their nests and a feeder.  
102 The routes were cleared from all vegetation that could interfere with ant observations  
103 and enclosed by thin white plastic planks (10 cm high) that were dug halfway into the  
104 ground. The slippery surface of the planks prevented ants foraging elsewhere while  
105 minimising the obstruction of surrounding views (Wystrach, Beugnon, & Cheng,  
106 2012). Ants could freely travel between nest and feeder, which was a ~ 15 × 15 × 15  
107 cm plastic bowl sunk into the ground that contained several kinds of sweet buttery  
108 biscuit crumbs. The walls of the bowl were covered with a thin layer of Fluon® and  
109 prevented ants from climbing. Ants that dropped into the feeder and picked up a  
110 crumb were marked individually with coloured acrylic or enamel paint (Tamiya™).  
111 During training, ants could leave the feeder via a small wooden ramp. Ants were  
112 considered trained and ready for testing once they had performed at least five  
113 foraging runs and were able to reach the feeder from the nest in a straight line  
114 (without colliding into any barriers). During tests (see below) the feeder ramp was  
115 removed to prevent other homing ants from interfering.

116

## 117 **Experiment 1**

118

119 Experiment 1 was conducted to assess the occurrence of peeking behaviour  
120 in backward walking ants across four different test conditions with one nest of  
121 *Catalgyphis velox* (summer 2017) located at the beginning of a straight 8.0 m × 1.8 m  
122 long foraging route. The length of the route was chosen to be long enough for ants to  
123 build up proper PI information but short enough to enable the ants to drag a crumb  
124 the whole distance without being too fatigued. Three thin (0.5 cm width), large  
125 wooden boards (2.4 m × 1.2 m) were connected (7.2 m × 1.2 m) and placed onto the  
126 foraging route (Fig. 1a, grey rectangles). These boards enhanced the tarsi grip of the  
127 ants and provided an even substrate that minimised potential interference with small  
128 grass haulms or pebbles during tests when ants dragged their food items backward.

129 During training, the individually marked foragers scuttled (forward) between  
130 the nest and feeder over the connected boards and familiarised themselves with the  
131 visual surroundings. After training, individual ants were subjected to one test  
132 conditions. All tests comprised of a forager that dragged a large biscuit crumb  
133 backward. For that, trained foragers with a small food item (~ 0.2 × 0.2 × 0.2 cm)  
134 were caught and transferred into a plastic vial. The food item was then carefully and  
135 manually removed and a larger biscuit piece (~ 2.0 × 0.5 × 0.2 cm) was offered to the  
136 ant instead. The biscuit provided was large enough to force the ants to drag it  
137 backward. After the ant locked mandibles onto the large biscuit, she was transferred  
138 to the appropriate release point. Four possible test conditions were carried out with  
139 either FV- (i.e., ants with their PI vector information, captured at the feeder) or ZV-  
140 ants (i.e., foragers without PI information, captured just before they enter the nest;  
141 Fig. 1a). To test whether ants pay attention to the visual scenery during backward  
142 motion, FV ants were released at the feeder either with the familiar scenery (FV,  
143 condition 1) or with altered visual surroundings (FVA, condition 2): by adding large

144 black plastic bags (~ 0.8 m × 0.6 m) on one side and a large dark tarp (0.9 m × 3.4  
145 m) on the other side of the route (Fig. 1a, right panel) to avoid potential obstructions.  
146 The objects were added a few seconds after the FVA-ant had started to home  
147 backward to ensure that the ant did not perceived any alteration before. To test the  
148 effect of route location, backward ants were released either at the beginning (ZV,  
149 condition 3) or at the middle of the route (ZVmid, condition 4).

150 It should be noted that upon release, the ants tested in Experiment 1 had the  
151 chance to glimpse forward while handling their large biscuit before engaging in  
152 backward movements.

153

#### 154 **Experiment 1: data and analysis**

155

156 For all tests, the distance between the release point and the location at which  
157 peeking behaviours occurred was noted with the help of a measuring tape placed on  
158 the side of the board that spanned from the feeder to the nest. Tests ended as soon  
159 as the backward walking ants reached the end of the wooden boards (i.e., ~0.5 m in  
160 front of the nest entrance) or abandoned her food item for more than one minute.

161 Individual ants were tested only once per test but were subjected to different test  
162 conditions with at least one un-interfered training trial between tests. The sequence  
163 of tests was evenly counter-balanced across individuals (e.g., FV-FVA-ZV-ZVmid;  
164 FVA-ZV-ZVmid-FV; ZV-ZVmid-FV-FVA etc.)

165 Comparison were made between FV- vs. FVA-ants and ZV- vs. ZVmid-ants  
166 (Fig. 1a). Given the large inter-individual variations, paired-data was applied and thus  
167 individuals that were not tested on both FV and FVA or ZV and ZVmid conditions  
168 were excluded from the analysis. We compared both the distance at which the first  
169 peeking behaviour occurred (1<sup>st</sup> peek distance) and the overall peek rate of



170 individuals (i.e., number of peek/distance walked) using Wilcoxon rank sum tests, a  
171 nonparametric statistic for paired data. Given that all ants walked rather straight  
172 toward the nest along the route, distance walked could be simply approximated by  
173 the beeline distance walked along the route. Most ants walked the full route (7.2 m)  
174 except obviously ants in the ZVmid condition and some foragers that abandoned  
175 their biscuit. For the comparison of peek rate, the 7.2 m long route on the boards was  
176 divided into half (Section 1: 0 - 3.4 m; Section 2: 3.4 - 7.2 m; Figure 1a). Thus, during  
177 ZVmid tests ants ran only Section 2. Comparisons between ZV- vs. ZVmid-ants were  
178 conducted to separate the effect of *distance walked* (i.e., ZVmid vs. ZV on Section 1)  
179 from the *actual location* along the route (i.e., ZVmid vs. ZV on Section 2). Bayesian  
180 statistics based on the Z values from these tests were applied to evaluate which of  
181 these alternative hypotheses explain peek rate best. We used  $f(Z)$ , that is,  
182 the y values on the standard normal distribution obtained from each of  
183 the Z scores, to estimate evidence in favour of each hypothesis. The ratio  
184 of the  $f(Z)$  measures of the two hypothesis gives an estimate of the 'evidence  
185 ratio'. While evidence ratios under 5 are weak, ratios over 10 are very strong (see  
186 Wystrach et al. 2014 for more details).

187         Backward paths were recorded by using GoPro HERO3+ cameras which  
188 were manually held approx. 0.6 m above the tested ant. Therefore, a quantification of  
189 the movement speed of the ants before and after peeking could be calculated. We  
190 measured the relative distance walked by the backward ants during the five seconds  
191 preceding the onset of the first peek (i.e., before the moment when the ants released  
192 the biscuit) and five seconds after the peek (i.e., after the ant resumed backward  
193 motion).

194

195 **Experiment 2**

196

197 Experiment 2 was conducted to test whether ants could guide their path  
198 backwards based on terrestrial cues. To do so, we recorded the paths of backward  
199 walking ants captured near their nest and released at four different release points  
200 (summer 2018). We used two different nests of *C. velox* ants. For each nest, a 5.0 m  
201 × 2.0 m straight foraging route was built with the nest entrance at one end and the  
202 feeder at the other end (Fig. 2a). As in Experiment 1, the route was enclosed by  
203 white plastic planks and ants were given a choice of biscuit crumbs inside the feeder  
204 to prompt foraging. However, here the ants scuttled back and forth directly on the  
205 natural ground during training. Once trained (see General methods), individual ants  
206 were captured on their way home 0.5 m before reaching their nest and subsequently  
207 released at one out of four possible locations (Fig. 2a):

208 Feeder (F): Ants were released 0.5 m after the feeder toward the nest direction.

209 Before nest (BeN): Ants were released on the route, 0.5 m before their nest.

210 Beyond nest (ByN): Ants were released 0.5 m beyond the nest in the feeder-nest  
211 direction.

212 Unfamiliar (U): Ants were released ~ 30.0 m away from the nest in a visually  
213 unfamiliar location.

214 For all tests, ants were captured in a plastic vial, offered a large biscuit crumb  
215 to incite backward walking and, once the ant had grabbed the cookie, released within  
216 a lampshade at the middle of a large wooden board (2.4 m × 1.2 m). Individual ants  
217 were tested only once per test, but could be subjected to different tests conditions,  
218 with at least one un-interfered training trial between tests. The wooden board was set  
219 in place just before and removed just after each test. The board was centred on the  
220 current release location with the long side along the feeder-nest direction (Fig. 2a) as  
221 it represents the expected homing direction. The board provided an even substrate

222 during tests and prevented ants to use potential familiar olfactory cues from the  
223 ground or the nest (BeN and ByN tests). A grid pattern (0.25 m × 0.25 m) drawn on  
224 the board enabled paths to be transcribed onto gridded paper. The lampshade (beige  
225 opaque fabric, 0.5 m diameter; 0.4 m height) surrounded the ant upon release and  
226 obstructed any familiar terrestrial views; the top of the lampshade was open providing  
227 the ant with celestial compass cues. Once the ant had pulled the large crumb  
228 backward for 0.1 m, the lampshade was lifted and the visual surrounding was  
229 revealed. This ensured that the ants could not utilise any familiar scenes before  
230 starting their backward path. This sets Experiment 2 apart from Experiment 1 where  
231 tested ants had the chance to glimpse forward before dragging the biscuit backward.

232

### 233 **Experiment 2: data and analysis.**

234

235 The backward paths and locations of peeking behaviour were noted. For each  
236 peek, the duration (i.e., the time the ant was not dragging the biscuit) was recorded.  
237 The actual trajectory of the path during forward motion was not recorded. Recording  
238 continued until the ants either reached the edge of the board or abandoned their  
239 large crumb for more than 1 min.

240 The recorded paths were digitised as (x, y) coordinates using GraphClick (Arizona  
241 Software). Path characteristics such as direction, meander and peek location were  
242 computed and analysed with Matlab™ (Mathworks, Matick, MA, USA). The meander  
243 index measures how much the path changes direction from segment to segment and  
244 was calculated as the mean angular deviation in direction between successive  
245 segments, each 0.3 m (Schwarz, Albert, Wystrach, & Cheng, 2011). Differences  
246 between test locations were determined using a generalised linear mixed effect  
247 model with repeated ants as random effect and Tukey's post hoc corrections. For

248 peek durations, a model for proportional (binomial) data was applied with 0 to 60 s  
249 (the duration at which recording was stopped) reported between 0 and 1.

250

## 251 **RESULTS**

252

### 253 **Experiment 1**

254

255 Experiment 1 was designed to investigate whether the occurrence of peeking  
256 behaviour in backward walking ants depends on factors such as: path integration,  
257 terrestrial cues, the location along the route or the distance walked.

258

#### 259 *FV vs. ZV: effect of path integration*

260 In both FV and ZV conditions, ants started to pull their biscuit toward the nest and  
261 mostly maintained that direction. However, the PI state had a visible effect on the  
262 occurrence of peeking behaviours: ZV-ants peeked earlier (first peek distance ZV-  
263 ants: mean  $\pm$  SD = 3.28  $\pm$  2.19 m; FV-ants: mean  $\pm$  SD = 5.90  $\pm$  1.93 m; Wilcoxon  
264 rank sum test:  $P < 0.001$ ,  $Z = -3.458$ ) and thrice as much (overall peek rate ZV-ants:  
265 mean  $\pm$  SD = 0.63  $\pm$  0.63 peek/m; FV-ants: mean  $\pm$  SD = 0.19  $\pm$  0.29 peek/m;  
266 Wilcoxon rank sum test:  $P < 0.001$ ,  $Z = -3.751$ ) than FV-ants (Fig. 1c, blue vs. green).  
267 Also, ZV-ants occasionally abandoned their large food item and did not resume  
268 backward movements (6 out of 33), whereas FV-ants never abandoned theirs (0 out  
269 of 32). A Fisher's exact test verified a significant difference ( $P = 0.032$ ). It seems  
270 clear that the lack (or conflicting) PI information decreases the ant's directional  
271 certainty.

272

#### 273 *FV vs. FVA: effect of altered visual familiarity*

274 To test the potential effect of the level of an altered visual scenery on backward  
275 walking ants, two conditions were conducted: (1) FV-ants homing backward on the  
276 unaltered, usual route, and (2) FVA-ants, homing backward on the same route but  
277 with altered visual surroundings (Fig. 1a). The alternation of the visual surrounding  
278 was effected only after the ants had started their journey backward, so that  
279 differences indicate that ants paid attention to terrestrial cues while walking  
280 backward. Results showed that FVA-ants peeked more often than FV-ants, however,  
281 this effect was weak, and reaches significance only in Section 2 (Wilcoxon rank sum  
282 test:  $P = 0.027$ ,  $Z = 3.751$ ) but not in Section 1 of the route (Wilcoxon rank sum test:  
283  $P = 0.688$ ,  $Z \sim 0$ ; Fig. 1d), due to a statistical floor effect. Indeed, a low rate of  
284 peeking in the first section of the route was expected, given that the path integration  
285 vector is longer and thus stronger at the beginning of the route home (Wystrach et  
286 al., 2015).

287 Second and most importantly, FVA-ants travelling in the altered visual  
288 environment displayed their first peek earlier along the route as compared to FV-ants  
289 on the familiar route (Wilcoxon rank sum test:  $P = 0.044$ ,  $Z = 2.016$ ; Fig. 1d).  
290 Together, this suggests that backward walking ants paid attention to terrestrial cues.

291 We also noted that, as for ZV-ants (see above), FVA-ants tested in the altered  
292 familiar condition abandoned their biscuits significantly more often than FV-ants  
293 (FVA: 6 out of 31 vs. FV: 0 out of 32; Fisher's exact test:  $P = 0.022$ ). Here again, it  
294 seems that the altered visual scenery decreases the directional certainty of backward  
295 walking ants.

296

297 *ZV vs. ZVmid: effect of location*

298 We investigated the potential effect of the location along the route by releasing  
299 zero vector ants either at the beginning (ZV) or in the middle of the familiar route

300 (ZVmid; Fig. 1a, c). Consequently, ZVmid-ants walked only Section 2, while ZV-ants  
301 moved along both sections. Ants displayed their first peek on average slightly earlier  
302 when released at the middle of the route (ZVmid) than when released at the  
303 beginning of the route (ZV; Wilcoxon rank sum test:  $P = 0.040$ ,  $Z = 2.062$ ; Fig. 1e).  
304 Also, the peek rate displayed by ZVmid-ants along Section 2 (the only section they  
305 walked) was higher than ZV-ants along Section 1 (Wilcoxon rank sum test:  $P =$   
306  $0.005$ ,  $Z = -2.814$ ) but similar to the peek rate displayed by these ZV-ants along  
307 Section 2 of the route (Wilcoxon rank sum test:  $P = 0.796$ ,  $Z = -0.2585$ ; Fig. 1e). A  
308 Bayesian evidence ratio (see Methods) was computed to estimate whether Section 1  
309 (i.e., different location, same distance walked) or Section 2 (i.e., same actual  
310 location, different distance walked) of ZV-ants' peek rate resembles most ZVmid-  
311 ants' peek rate. The obtained evidence ratio was 50.74 in favour of Section 2, which  
312 equals 'overwhelming evidence' for an effect on peek rate of the actual location along  
313 the route rather than the distance walked.

314

### 315 *Peeking and walking speed*

316 Interestingly, in all conditions, and for the vast majority of the individuals, ants walked  
317 backward on average twice as quickly after peeking than before peeking (Fig. 1b).  
318 This supports the idea that a peeking event increases the ant's directional certainty  
319 for some time.

320

## 321 **Experiment 2**

322

323 Experiment 2 was conducted to test whether ants could guide their path  
324 backwards using terrestrial cues. To do so, we recorded the paths of backward  
325 walking ants captured near their nest and released at four different release points.

326 Unlike as in Experiment 1 all tested ants in Experiment 2 were prevented from  
327 monitoring the visual surrounding before dragging their food item backward as a  
328 lampshade was blocking the whole panoramic view upon release (see Methods).  
329 Hence, any difference in the taken direction must result from visual information  
330 perceived while the ants were dragging their crumb backward – at least until they  
331 peeked for the first time.

332

### 333 *Ants can guide their backward path*

334 To see whether ant paths were directed in different direction across release  
335 locations, the Y-values – the end position along the feeder-to-nest line relative to the  
336 release point – were examined. The Y-values at the end of the foragers' path varied  
337 across released location (ANOVA:  $F = 21.96$ ,  $P < 0.001$ ; Fig. 2c). Ants released at  
338 the feeder were directed toward the nest and hence obtained the highest Y-values  
339 (Tukey's post-hoc test F vs. BeN, ByN and U:  $Z_s > 3.75$ ,  $P_s < 0.001$ ; Fig. 2c). Ants in  
340 unfamiliar tests (U) showed no directional preference along the Y-axis (Fig. 2c), as  
341 expected given the lack of familiar visual information at this location. Interestingly,  
342 ants from BeN and ByN conditions differed significantly in their final Y-values on the  
343 board (Tukey's post-hoc test BeN vs. ByN:  $Z = 3.47$ ,  $P = 0.003$ ). The medians of both  
344 of these groups are close to the actual nest location, showing that they used familiar  
345 visual cues to search at the nest (Fig. 2c). Differences between conditions could also  
346 be observed in path meander (ANOVA:  $F = 9.07$   $P < 0.001$ ). Ants from the feeder  
347 test showed straighter paths than ants from all other conditions (Tukey's post-hoc  
348 test F vs. BeN, ByN and U:  $Z_s > 3.68$   $P_s < 0.002$ ; Fig. 2c). No difference in meander  
349 among the remaining test conditions could be determined. Indeed, BeN-, ByN- and  
350 U-ants were expected to search on the board: BeN- and ByN-ants due to the  
351 proximity of the nest and U-ants due to the of the lack of familiar visual information.

352 Overall, these data show that ants could use familiar visual cues to adequately direct  
353 their backward paths.

354 Remarkably, analysis of the paths displayed purely backwards, before the first  
355 peek (or until the ant left the board if she did not peek), showed a similar pattern of  
356 results for both the Y-values reached (ANOVA:  $F = 11.37$   $P < 0.001$ ) and path  
357 meander (ANOVA:  $F = 3.52$   $P = 0.024$ ) (Fig. 2D). Despite purely backward  
358 movements, ants released at the feeder travelled significantly longer distances along  
359 the feeder-nest direction (Tukey's post-hoc test: F vs. BeN, ByN and U:  $Z_s > 3.29$   $P_s$   
360  $< 0.006$ ; Fig. 2d) and displayed straighter paths (Tukey's post-hoc F vs. ByN and U:  
361  $Z_s > 2.65$   $P_s < 0.03$ ; F vs. BeN:  $Z = 1.88$   $P = 0.235$ ; Fig. 2d) before peeking. The  
362 three other groups (BeN, ByN, U) were indeed expected to search on the board and  
363 to perform a similar level of path meander, as observed. Differences in the Y-values  
364 reached when released on each side of the nest did not reach significance using  
365 Tukey's post-hoc test (BeN vs. ByN:  $Z = 2.084$ ,  $P = 0.158$ ), however, the pattern of  
366 results followed what was expected if ants were using views to direct their path  
367 toward the nest. Ants released before (BeN) and beyond (ByN) their nest both moved  
368 on average toward the nest location, that is, in opposite direction from their release  
369 points; and ants released at the unfamiliar test site (U) showed less directed paths  
370 (Fig. 2d). The differences in paths characteristics is reflected if one considers the  
371 probability of obtaining the expected order of path endpoint across the four test  
372 conditions (Y-value:  $F > \text{BeN} > \text{U} > \text{ByN}$ ) is  $1/4! = 0.042$ .

373 Because nest-directed path sections were achieved before the ants triggered their  
374 first peek and the visual panorama was revealed to them only after they had started  
375 backward motion, the differences across locations show that ants can recognise and  
376 use the familiar visual cues to guide their path while moving backward and without  
377 the need of peeking.



378 Interestingly, several ants released at the feeder (4 out of 16 ants) displayed nest-  
379 directed backward paths across the whole recording board without performing a  
380 single peek and by keeping their body orientation away from the feeder-nest direction  
381 by at least 90° (bold paths in Fig. 2d).

382

### 383 *Peek duration and past information*

384 We also tested whether peek duration was influenced by the test condition and the  
385 number of previously displayed peeks (Fig. 2b). The feeder condition was excluded  
386 from this analysis as these ants were expected to move in a straight line and exit the  
387 board so that the actual peek number of a given ant may correlate with the location  
388 where the ant peeks. The three other groups (BeN, ByN, U) on the other hand, are  
389 expected to search on the board so any effect of the peek number is unlikely to be  
390 attributed to a specific location on the board. Interestingly, peek duration, which was  
391 recorded up to 60 s, was strongly influenced by the number of peeks previously  
392 displayed by the ant (GLM peek number:  $F = 17.09$ ,  $P < 0.001$ ; Fig. 2b) and not the  
393 actual test condition (GLM condition:  $F = 0.17$ ,  $P = 0.841$ ; Fig. 2b). The more peeks  
394 an ant had previously displayed the longer its current peeking lasts. This shows that  
395 the ant's peeking behaviour is also modulated by past information. Whether it is the  
396 time passed or the number of peek previously displayed cannot be disentangled  
397 here.

398

## 399 **DISCUSSION**

400

401 Ants dragging a heavy food item backward occasionally trigger a so-called  
402 'peeking behaviour' or 'peek': ants drop their food and turn around to look forward.  
403 Aligning their body in a familiar direction enables them to recognise the learnt visual

404 panorama and store this direction to follow it during their subsequent backward path  
405 (Schwarz et al., 2017). It is clear that ants gain directional information from learnt  
406 terrestrial cues when peeking forward. However, whether or not they use terrestrial  
407 cues while dragging their food item backward is less clear. Several of the current  
408 results demonstrate that ants are indeed able to do so, raising question about the  
409 underlying mechanisms.

410

### 411 **Ants still recognise terrestrial cues while walking backward**

412

413 Experiment 1 shows that the visual scenery experienced while walking backward  
414 influences the occurrence of peeking behaviour. First, zero vector (ZV) ants (i.e., ants  
415 captured at the nest and thus deprived of useful path integration (PI) information),  
416 displayed their first peek earlier when starting their backward journey halfway along  
417 the route than when starting at the beginning of the route (Fig. 1e). Second, full  
418 vector (FV) ants (i.e., captured at the feeder and thus having a PI vector pointing  
419 towards the nest) displayed their first peek earlier along the route if the surrounding  
420 scenery was artificially altered (FVA, Fig. 1d). This was true even though the scene  
421 was manipulated only after the ants had started dragging their biscuit backward  
422 indicating that ants paid attention to the familiar scenery while walking backward. It  
423 should be noted that this effect was weak (Fig. 1d), possibly because the alteration of  
424 the scene was not obvious enough (Schwarz et al., 2014).

425 In Experiment 2, ants could guide their trajectories based on terrestrial cues while  
426 walking backward. Ants were released on a board (ruling out the use of olfactory  
427 cues) and within a lampshade at different location relative to the nest (Fig. 2). The  
428 visual world was revealed to them only after they had started their backward journey.  
429 Nonetheless and despite the lack of PI homing vector, their paths were oriented in

430 the expected direction (i.e., the nest) resulting in differences between test conditions.  
431 Importantly, this was also true for the portion of path displayed before their first peek,  
432 that is, displayed purely backward (Fig. 2d).

433 In sum, ants can use learnt terrestrial visual cues while walking backward to guide  
434 their path as well as decide whether and when to peek forward. The next section  
435 discusses potential explanations.

436

### 437 **Mental rotation or combining attractive and repulsive views?**

438

439 How can ants recognise views backward? This is a puzzling question given that  
440 the assumption of current models of visual homing states that views must be  
441 retinotopically aligned to provide directional information (Ardin, Peng, Mangan,  
442 Lagogiannis, & Webb, 2016; Baddeley et al., 2012; Collett, Graham, & Collett, 2017;  
443 Le Möel & Wystrach, 2019; Möller, 2012; Murray et al., 2020; Narendra et al., 2013;  
444 Wystrach, Cheng, et al., 2011; Wystrach, Mangan, Philippides, & Graham, 2013;  
445 Zeil, Hofmann, & Chahl, 2003) although some other processes may be also at work  
446 (Wystrach et al., 2012). Recently, it has been suggested that ants may perform some  
447 sort of mental rotation to compare misaligned views (Ardin, Mangan, Wystrach, &  
448 Webb, 2015; Ardin et al., 2016), which may be achieved if views are encoded in the  
449 frequency domain (Stone et al., 2017). However, one can wonder why, if they can  
450 achieve mental rotation, would the ants peek at all? A capacity for mental rotation is  
451 hard to reconcile with the result of previous experiments where ants would not adjust  
452 their backward trajectory at all unless they peeked to align their body in the correct  
453 direction (Schwarz et al., 2017).

454 An alternative hypothesis would be that backward dragging ants may assess the  
455 maximum, rather the minimum of the rotational image difference function (Zeil et al.,

456 2003). However, this fails to explain why ants succeed to use terrestrial cues to guide  
457 their path backward on a two-way route but not on a one-way route (Schwarz et al.,  
458 2017). Also, to assess the maximum (as for the minimum (Wystrach et al., 2015)) for  
459 homing requires ants to scan the world to evaluate the relative mismatch across the  
460 different rotations; here however, the ants could maintain a straight backwards path  
461 without scanning (Fig. 2d).

462 Here we suggest instead a third hypothesis: ants may still need to align their body  
463 in the familiar direction to recognise memorised views but possess a memory bank of  
464 views learnt while facing in multiple directions and not only toward the nest – as  
465 suggested in a related manner concerning learning- and orientation walks (Zeil &  
466 Fleischmann, 2019). Notably, views learnt while facing in the anti-nest direction could  
467 be treated as repulsive when homing (Fig. 3a). The familiarities resulting from the  
468 comparison of the currently perceived view with both attractive (nest facing) and  
469 aversive (feeder facing) visual memories could simply be compared in a way  
470 somewhat analogous to an opponent process, as recently modelled in the light of the  
471 insect's neural circuitry (Le Möel & Wystrach, 2019). The signal resulting from this  
472 comparison informs the ant about whether to move toward or away from the currently  
473 faced direction, without the need for scanning (Le Möel & Wystrach, 2019). The only  
474 additional assumption needed for this model to explain backward guidance is that, if  
475 the ant is holding a large food item, 'moving away' means going backward because  
476 the ant cannot lift the cookie to turn its body. Fig. 3a illustrates the functionality of this  
477 simple model.

478 In the light of this model, we can wonder whether ants on the way home can also  
479 use the visual memories stored during their outbound trips (i.e., when they went from  
480 the nest to the feeder) as repulsive. This idea challenges the opinion that ants treat  
481 in- and outbound trip visually separately depending on the motivational state (Harris,

482 Hempel de Ibarra, Graham, & Collett, 2005; Wehner, Boyer, Loertscher, Sommer, &  
483 Menzi, 2006). Instead, ants may always recall both their memorised inbound and  
484 outbound facing views but treat them as repulsive or attractive depending on their  
485 current motivational state.

486

487 This attraction/repulsion model explains several observed phenomena of the  
488 current and past studies. (1) In a former experiment (Schwarz et al., 2017) backward  
489 ants were not able to correct their path at all while walking backward because, in this  
490 particular set-up, in- and out-bound routes were spatially separated (as a one-way  
491 circuit) so that no outbound views were available to potentially help out backward  
492 homing ants. (2) In the current Experiment 2, backward ants released at the feeder  
493 (F) carried on in the correct nest direction (Fig. 2d) because they recognised  
494 outbound views oriented toward the feeder, driving them away from (or opposite to)  
495 this direction (Fig. 3a). (3) Alteration of the visual surrounding would trigger earlier  
496 peeking behaviours because the familiarity of the feeder facing (outbound) views  
497 would be equally altered, disrupting the repulsive effect and thus reducing the overall  
498 directional drive (Fig. 1d). (4) Assuming that outbound views near the feeder are  
499 more familiar than in the middle of the route (ants perform learning walks at the  
500 feeder: (Nicholson, Judd, Cartwright, & Collett, 1999), the repulsive effect would be  
501 stronger for ants released at the feeder than in the middle of the route, yielding to a  
502 stronger directional drive and hence less peeking near the feeder (Fig. 1d). (5)  
503 Further, it was surprising in Experiment 2 that ants released close to the nest could  
504 direct their backward paths toward the nest (BeN, ByN; Figure 2c, d). Although this  
505 verifies that they recognised familiar views they nonetheless tended to peek often  
506 and even abandoned their cookie close to the nest (9 out of 28 in BeN- and 4 out of  
507 20 in ByN-test). This seems counter-intuitive, yet it can be explained in the light of the

508 integration between 'attractive and 'repulsive view'. During learning walks around the  
509 nest, ants appear to store indeed both nest and anti-nest oriented views (Jayatilaka,  
510 Murray, Narendra, & Zeil, 2018). Even if these nest views may be all extremely  
511 familiar, the integration of attractive (nest-oriented) and repulsive (anti-nest oriented)  
512 views would result in a low overall directional drive, and thus lead to high peek rates  
513 (and a high probability for abandoning the crumb) but nonetheless guide the ants  
514 toward the nest. This very same idea explained the recent puzzling observation that  
515 ants behaved similarly at the nest and in unfamiliar settings, but not on the foraging  
516 route (Murray et al., 2020). (6) Finally, two recent studies revealed the importance of  
517 outbound trips for homing ants. Ants with outbound views during training were more  
518 efficient in homing than ants with only inbound views during training (Freas & Cheng,  
519 2018; Freas & Spetch, 2019). But whether homing ants actually used their outbound  
520 view as suggested here, or simply learnt homing views by turning back during their  
521 outbound trips remains to be seen.

522

523 **Ants combine multiple cues to estimate directional uncertainty and trigger**  
524 **peeks.**

525

526 It is known that ants combine the directional dictates of the current visual  
527 familiarity with their PI in a weighted fashion (Wehner, Hoinville, Cruse, & Cheng,  
528 2018). Notably, the direction indicated by the current view is more weighted as the  
529 current view is familiar (Legge et al., 2014) and the direction indicated by the PI is  
530 more weighted as the PI vector length increases (Wystrach et al., 2015). Backward  
531 walking ants appear to weight these cues in the same fashion. Fig. 3b shows how  
532 such an integration of cues captures the peek rate observed across our conditions  
533 and distance walked along the route. Notably, this explains why ZV-ants peeked

534 earlier and more often than FV-ants (Fig. 1b), as observed in North African  
535 *Cataglyphis fortis* ants (Pfeffer & Wittlinger, 2016) and why peek rate increases as  
536 the distance walked along the route increases (Fig. 1d, e), as observed in *Myrmecia*  
537 *croslandi* ants (see Fig. S2 in Schwarz et al. 2017).

538 Interestingly, such an estimate of directional certainty seems not only to influence  
539 the occurrence of peeking but also whether the peeking ants decided to return to  
540 their biscuit or abandon it. FV-ants in familiar visual surroundings and therefore with  
541 the highest directional certainty, dragged their biscuit all along the 7.20 m route  
542 without exception (32 out of 32). In contrast, some ants abandoned their biscuits in  
543 both FVA (6 out of 31) and ZV (6 out of 33) conditions.

544 Finally, it is worth mentioning that ants clearly increased the speed of their  
545 backward motion after peeking (Fig. 1c). The increase in speed is likely not only a  
546 mere consequence of a short recovery from the dragging activity but also a gain in  
547 navigational certainty as this sudden speed increase is also apparent when displaced  
548 foragers reach their familiar route and recognise the familiar scenery (pers. observ.).  
549 Furthermore, a decrease in speed when ants run off their PI has been described  
550 (Buehlmann, Fernandes, & Graham, 2018). Hence it seems that the speed of  
551 movement reflects the strength of the directional drive and therefore directional  
552 certainty. If peeking behaviour seems to increase directional certainty, the later seem  
553 to decrease with time spent backwards (Fig. 3c), which also make functional sense.

554

#### 555 **Ants gather information about the time spent backward.**

556

557 Recently, it has been shown that directional information based on terrestrial cues is  
558 obtained when ants face forward during peeks and must therefore be stored in a  
559 short-term memory while the ant is subsequently walking backward (Schwarz et al.,

2017). Our results suggest that short-term memory also influences the ants' navigational certainty. The time spent peeking forward varied (as already noted by Pfeffer and Wittlinger (2016)) with some ants 'peeking' for less than a second while other spent more than 60s (after which recording was stopped) without returning to their cookie. Interestingly, the more an ant had peeked before during a test run, the longer her current peek lasted (Fig. 2b). This shows that ants somehow gather information across time to adjust their current certainty: either information about the overall time spent backward or information about the number of peek previously displayed (see also Fig. 3c).

Former experiments have already shown that the behaviour of ants can be modulated by recent experience in the order of seconds to minutes – a form of hysteresis (Graham & Mangan, 2015). For instance, ants display a so-called backtracking behaviour only if they have recently perceived the nest surrounding (Wystrach, Schwarz, Baniël, & Cheng, 2013). Also, homing ants display higher meander in their paths when recapitulating a well-known route for the second time in a row (Collett, 2014; Wystrach, Schwarz, Graham, & Cheng, 2019). Finally, ants can remember the compass direction of a wind gust after being blown (Wystrach & Schwarz, 2013). In the present case, ants seem to also build up information about the recent past. Even though it makes functional sense it remains to be seen what neural or physiological mechanisms underlie this phenomenon.

580

## 581 **Conclusion**

582 This study confirmed that ants walking backward are not just paying attention to  
583 celestial cues but combine multiple information from their PI, the recognition of  
584 terrestrial cues and temporal information such as the time they spent backward. All  
585 this information seems to be integrated in an overall directional drive. This drive,



586 which reflects the current directional certainty, seems to (1) guide the ants backward  
587 path, (2) triggers peeking behaviour if too low and (3) finally dictates whether or not  
588 to return to their food item during peeks. Importantly, this study shows that ants uses  
589 familiar terrestrial cues while navigating backward. In addition to the attractive  
590 memories facing toward the nest, the hypothesis that homing ants use a collection of  
591 repulsive visual memories facing away from the nest and possibly stored during their  
592 outbound trip was put forward (Le Möel & Wystrach, 2019). In the light of this  
593 hypothesis, visual navigation forward or backward can then simply be achieved by  
594 using the relative familiarity between both sets of opposite valence memories. As  
595 often with research on insect navigation, remarkably flexible behaviours incite  
596 researchers to endorse the simplest explanations (Wystrach & Graham, 2012).

597

#### 598 **Author contributions**

599 Conceptualisation: S.S., A.W.; Methodology: S.S., A.W.; Formal analysis: S.S., A. W.  
600 Investigation: S.S., L.C., E.G., A.W.; Writing - original draft: S.S.; Writing - review &  
601 editing: S.S., L.C., E.G., A.W.; Funding: A.W.

602

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611 **References**

612

613 Ardin, P., Mangan, M., Wystrach, A., & Webb, B. (2015). How variation in head pitch  
614 could affect image matching algorithms for ant navigation. *Journal of*  
615 *Comparative Physiology A*, 201, 585–597.

616 Ardin, P., Peng, F., Mangan, M., Lagogiannis, K., & Webb, B. (2016). Using an insect  
617 mushroom body circuit to encode route memory in complex natural  
618 environments. *PLoS Computational Biology*, 12, e1004683.

619 Ardin, P. B., Mangan, M., & Webb, B. (2016). Ant homing ability is not diminished  
620 when traveling backwards. *Frontiers in Behavioral Neuroscience*, 10:69.

621 Baddeley, B., Graham, P., Husbands, P., & Philippides, A. (2012). A Model of Ant  
622 Route Navigation Driven by Scene Familiarity. *PLoS Computational Biology*,  
623 8, 1002336.

624 Buehlmann, C., Fernandes, A., & Graham, P. (2018). The interaction of path  
625 integration and terrestrial visual cues in navigating desert ants: what can we  
626 learn from path characteristics? *The Journal of Experimental Biology*, 221.

627 Cerda, X. (2001). Behavioural and physiological traits to thermal stress tolerance in  
628 two Spanish desert ants. *Etologia*, 9, 15-27.

629 Cheng, K., Narendra, A., Sommer, S., & Wehner, R. (2009). Traveling in clutter:  
630 navigation in the Central Australian desert ant *Melophorus bagoti*. *Behavioural*  
631 *Processes*, 80, 261-268.

632 Collett, M. (2012). How navigational guidance systems are combined in a desert ant.  
633 *Current Biology*, 22, 927-932.

634 Collett, M. (2014). A desert ant's memory of recent visual experience and the control  
635 of route guidance. *Proceedings of the Royal Society B: Biological Sciences*,  
636 281, 20140634.

637 Collett, M., Graham, P., & Collett, T. S. (2017). Insect navigation: what backward  
638 walking reveals about the control of movement. *27*, 141-143.

639 Collett, T., Graham, P., & Durier, V. (2003). Route learning by insects. *Current*  
640 *Opinion in Neurobiology*, *13*, 718-725.

641 Freas, C. A., & Cheng, K. (2018). Landmark learning, cue conflict, and outbound  
642 view sequence in navigating desert ants. *Journal of Experimental Psychology:*  
643 *Animal Learning and Cognition*, *44*, 409-421.

644 Freas, C. A., & Spetch, M. L. (2019). Terrestrial cue learning and retention during the  
645 outbound and inbound foraging trip in the desert ant, *Cataglyphis velox*.  
646 *Journal of Comparative Physiology A*, *205*, 177-189.

647 Graham, P., & Mangan, M. (2015). Insect navigation: do ants live in the now? *The*  
648 *Journal of Experimental Biology*, *218*, 819-823.

649 Harris, R. A., Hempel de Ibarra, N., Graham, P., & Collett, T. S. (2005). Priming of  
650 visual route memories. *Nature*, *438*, 302.

651 Heinze, S., Narendra, A., & Cheung, A. (2018). Principles of insect path integration.  
652 *Current Biology*, *28*, 1023-1058.

653 Hoinville, T., & Wehner, R. (2018). Optimal multiguide integration in insect  
654 navigation. *Proceedings of the National Academy of Sciences of the United*  
655 *States of America*, *115*(11), 2824-2829.

656 Jayatilaka, P., Murray, T., Narendra, A., & Zeil, J. (2018). The choreography of  
657 learning walks in the Australian jack jumper ant *Myrmecia croslandi*. *The*  
658 *Journal of Experimental Biology*, *221*, 185306.

659 Le Möel, F., & Wystrach, A. (2019). Opponent processes in visual memories: a  
660 model of attraction and repulsion in navigating insects' mushroom bodies.  
661 *bioRxiv*. doi:10.1101/2019.12.16.877449

662 Legge, E., Wystrach, A., Spetch, M., & Cheng, K. (2014). Combining sky and earth:  
663 desert ants (*Melophorus bagoti*) show weighted integration of celestial and  
664 terrestrial cues. *The Journal of Experimental Biology*, 217, 4159-4166.

665 Mangan, M., & Webb, B. (2012). Spontaneous formation of multiple routes in  
666 individual desert ants (*Cataglyphis velox*). *Behavioral Ecology*, 23, 944-954.

667 Möller, R. (2012). A model of ant navigation based on visual prediction. *Journal of*  
668 *Theoretical Biology*, 305, 118-130.

669 Murray, T., Zoltán, K., Dahmen, H., Le Möel, F., Wystrach, A., & Zeil, J. (2020). The  
670 role of attractive and repellent scene memories in ant homing (*Myrmecia*  
671 *croslandi*). *The Journal of Experimental Biology*, 223, jeb210021.

672 Narendra, A., Gourmaud, S., & Zeil, J. (2013). Mapping the navigational knowledge  
673 of individually foraging ants, *Myrmecia croslandi*. *Proceedings of the Royal*  
674 *Society B: Biological Sciences*, 280, 20130683.

675 Nicholson, D. J., Judd, S. P. D., Cartwright, B. A., & Collett, T. S. (1999). Learning  
676 walks and landmark guidance in wood ants (*Formica rufa*). *The Journal of*  
677 *Experimental Biology*, 202, 1831-1838.

678 Pfeffer, S., Wahl, V., & Wittlinger, M. (2016). How to find home backwards?  
679 Locomotion and inter-leg coordination during rearward walking of *Cataglyphis*  
680 *fortis* desert ants. *The Journal of Experimental Biology*, 219, 2110-2118.

681 Pfeffer, S., & Wittlinger, M. (2016). How to find home backwards? Navigation during  
682 rearward homing of *Cataglyphis fortis* desert ants. *The Journal of*  
683 *Experimental Biology*, 219, 2119-2126.

684 Reid, S. F., Narendra, A., Hemmi, J. M., & Zeil, J. (2011). Polarised skylight and the  
685 landmark panorama provide night-active bull ants with compass information  
686 during route following. *The Journal of Experimental Biology*, 214, 363-370.

687 Schwarz, S., Albert, L., Wystrach, A., & Cheng, K. (2011). Ocelli contribute to the  
688 encoding of celestial compass information in the Australian desert ant  
689 *Melophorus bagoti*. *The Journal of Experimental Biology*, 214, 901-906.

690 Schwarz, S., Julle-Daniere, E., Morin, L., Schultheiss, P., Wystrach, A., Ives, J., &  
691 Cheng, K. (2014). Desert ants (*Melophorus bagoti*) navigating with robustness  
692 to distortions of the natural panorama. *Insectes Sociaux*, 61, 371-383.

693 Schwarz, S., Mangan, M., Zeil, J., Webb, B., & Wystrach, A. (2017). How Ants Use  
694 Vision When Homing Backward. *Current Biology*, 27, 401-407.

695 Stone, T., Webb, B., Adden, A., Weddig, N., Honkanen, A., Templin, R., . . . Heinze,  
696 S. (2017). An anatomically constrained model for path integration in the bee  
697 brain. *Current Biology*, 27, 3069-3085.e3011.

698 Wehner, R. (2003). Desert ant navigation: how miniature brains solve complex tasks.  
699 *Journal of Comparative Physiology A*, 189, 579-588.

700 Wehner, R., Boyer, M., Loertscher, F., Sommer, S., & Menzi, U. (2006). Ant  
701 navigation: one-way routes rather than maps. *Current Biology*, 16, 75-79.

702 Wehner, R., Hoinville, T., Cruse, H., & Cheng, K. (2018). Steering intermediate  
703 courses: desert ants combine information from various navigational routines.  
704 *Journal of Comparative Physiology A*, 202, 459–472.

705 Wehner, R., & Srinivasan, M. V. (2003). Path integration in insects. In K. K. Jeffrey  
706 (Ed.), *The neurobiology of spatial behaviour* (pp. 9-30). Oxford: Oxford  
707 University Press.

708 Wittlinger, M., Wehner, R., & Wolf, H. (2006). The ant odometer: stepping on stilts  
709 and stumps. *Science*, 312, 1965-1966.

710 Wystrach, A., Beugnon, G., & Cheng, K. (2011). Landmarks or panoramas: what do  
711 navigating ants attend to for guidance? *Frontiers in Zoology*, 8, 21.

712 Wystrach, A., Beugnon, G., & Cheng, K. (2012). Ants might use different view-  
713 matching strategies on and off the route. *The Journal of Experimental Biology*,  
714 *215*, 44-55.

715 Wystrach, A., Cheng, K., Sosa, S., & Beugnon, G. (2011). Geometry, features, and  
716 panoramic views: Ants in rectangular arenas. *Journal of Experimental*  
717 *Psychology: Animal Behavior Processes*, *37*, 420-435.

718 Wystrach, A., & Graham, P. (2012). What can we learn from studies of insect  
719 navigation? *Animal Behaviour*, *84*, 13-20.

720 Wystrach, A., Mangan, M., Philippides, A., & Graham, P. (2013). Snapshots in ants?  
721 New interpretations of paradigmatic experiments. *The Journal of Experimental*  
722 *Biology*, *216*, 1766-1770.

723 Wystrach, A., Mangan, M., & Webb, B. (2015). Optimal cue integration in ants.  
724 *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20151484.

725 Wystrach, A., & Schwarz, S. (2013). Ants use a predictive mechanism to compensate  
726 for passive displacements by wind. *Current Biology*, *23*, 1083-1085.

727 Wystrach, A., Schwarz, S., Baniel, A., & Cheng, K. (2013). Backtracking behaviour in  
728 lost ants: an additional strategy in their navigational toolkit. *Proceedings of the*  
729 *Royal Society B: Biological Sciences*, *280*, 20131677.

730 Wystrach, A., Schwarz, S., Graham, P., & Cheng, K. (2019). Running paths to  
731 nowhere: repetition of routes shows how navigating ants modulate online the  
732 weights accorded to cues. *Animal Cognition*, *22*, 213–222.

733 Wystrach, A., Schwarz, S., Schultheiss, P., Baniel, A., & Cheng, K. (2014). Multiple  
734 sources of celestial compass information in the Central Australian desert ant  
735 *Melophorus bagoti*. *Journal of Comparative Physiology A*, *200*, 591-601.

736 Zeil, J. (2012). Visual homing: an insect perspective. *Current Opinion in*  
737 *Neurobiology*, *22*, 285–293.

738 Zeil, J., & Fleischmann, P. N. (2019). The learning walks of ants (Hymenoptera:  
739 Formicidae). *Myrmecological News*, 29, 93-110.

740 Zeil, J., Hofmann, M. I., & Chahl, J. S. (2003). Catchment areas of panoramic  
741 snapshots in outdoor scenes. *Journal of the Optical Society of America A*, 20,  
742 450-469.

743

744

745 **Figure 1.** Dynamics of peeking behaviours in terms of path integration and visual  
746 familiarity (Experiment 1). (a) Schematics of experimental set-up with training and  
747 test conditions. During training ants foraged between nest and feeder (~ 8.0 m) on  
748 three thin wooden boards serving as an even substrate (grey rectangles). The route  
749 was divided into two sections corresponding to the first and second half of the route.  
750 For tests, trained ants were either captured at the feeder (full-vector ants, FV) or just  
751 before entering the nest after foraging (zero-vector ants, ZV; open crosses) and  
752 released at the feeder (as FV- FVA or ZV-ants) or on the middle of the route (ZVmid-  
753 ants; black crosses). For FVA (altered familiarity), the familiarity of the route was  
754 manipulated by adding large black visual objects (black blobs) on one side and a  
755 dark tarp (black vertical bar) on the other side of the route. Dashed lines depict  
756 example paths of the ants. (b) Change in speed after the first peek. Each dot shows  
757 the relative change in speed (5s after/5s before) the first peek for each ant. Dotted  
758 line at 1 indicates no change in speed. (c) Cumulated number of peeks displayed  
759 against the distance walked along the route (mean  $\pm$  SE across individuals). (d)  
760 Overall peek rate (left) and distance of the first peek (right) for both FV-ant and FVA-  
761 ants. 7.3 m indicates the end of the route. (e) Overall peek rate (left) and distance of  
762 the first peek (right) for both ZV-ant conditions. 3.8 m indicates the end of the route  
763 (ZV-ant paths were truncated at 3.8 m to match the maximum homing distance of  
764 ZVmid-ants). Grey lines (d, e) represent individually tested ants across conditions.  
765 See main text for statistical details.

766

767 **Figure 2.** Backward path characteristics and peeking behaviour at different release  
768 points (Experiment 2). (a) Schematics of experimental set-up with training and test  
769 conditions. During training ants foraged between nest and feeder (~ 5.0 m). Trained  
770 ants with a remaining homing vector of 0.5 m (open cross depicts capture point) were



771 tested backward at different release points: feeder (F), before nest (BeN), beyond  
772 nest (ByN) and unfamiliar (U) test site. Ants were released (black crosses) on the  
773 middle of a thin wooden board (grey) to rule out the use of olfactory cues. Boards  
774 had a 0.25 m × 0.25 m squared pattern to enable path recording. Dashed lines depict  
775 example paths of ants during training. (b) Duration of peek (the time the ant spent  
776 away from the cookie) as a function of peek number for each individual. Max.  
777 threshold of peek duration was set to 60s and 'Feeder condition' was removed from  
778 this analysis as peek number correlates with position along the board. (c) Complete  
779 recorded backward paths of ants across conditions. Paths ended either because ants  
780 left the board or abandoned their cookie (peek duration > 60s). Boxplots show  
781 distance reached at the end of the path along the feeder nest axis (Y axis) and  
782 meander of the paths across individuals. Differences in top letters (a, b, c) indicate  
783 significant differences between groups (alpha = 0.05). Black dotted lines depict  
784 release point, black arrows remaining vector length (0.5 m) and open stars actual  
785 nest position. (d) As in (c), except that paths were truncated at the first peek or when  
786 exiting the board. Hence all navigational information gathered by ants was obtained  
787 while walking backward. Thick paths (left panel) emphasise four ants in the feeder  
788 conditions that displayed nest-directed backward paths without facing the nest  
789 direction. Open circles represent positions of 1<sup>st</sup> peeks. For statistical details see text.  
790

791 **Figure 3.** Models on how ants may combine navigational information. The longer the  
792 length of the drawn vectors the higher the directional drive. Dots represent cases with  
793 no directional drive and stars nest locations. (a) Illustration of the 'repulsive view  
794 hypothesis'. Overall directional drive results from the integration of attractive homing  
795 views (green arrows) minus repulsive outbound views (red arrows). Left scheme  
796 represents theoretical positions and orientations of memorised views. The ant

797 recognises only views that are aligned with its current body orientation (here, always  
798 facing downward). The larger the distance from the current location to the closest  
799 aligned view, the lower the familiarity and directional drive. This principle provides  
800 appropriate guidance toward the nest. Right scheme shows examples of integration  
801 for different locations (grey dashed arrows) with the ant always facing downward. For  
802 instance, when facing downward at the BeN location, the closest aligned view is  
803 repulsive (bold red arrow on right left scheme). Given that the neighbouring green  
804 arrow is not aligned, the closest aligned attractive view is further away, beyond the  
805 nest (bold green arrow on left scheme). Overall, the ants at this position (still facing  
806 downward) will be more repulsed than attracted by the current facing direction and  
807 thus walk backward toward the nest. In contrast, when beyond the nest ByN (still  
808 facing downward), the attractive views will match better than the repulsive views and  
809 the ants will thus turn around and walk backward toward the nest. Whatever the  
810 position and orientation of the ants around the nest, the agent will be drawn towards  
811 the nest (b) Directional drive across test conditions of Experiment 1 in Section 1 and  
812 2. Ants are tested in FV (full-vector), FVA (full-vector altered familiarity) and ZV (zero-  
813 vector) conditions. Overall directional drive (dark blue vectors) results from the  
814 integration of path integration (cyan vectors, the longer the path integration vector the  
815 stronger its directional drive) and learnt view (yellow vectors, the more familiar the  
816 view the stronger its directional drive). (c) Directional drive resulting from the  
817 recognition of a learnt view (yellow vectors) decrease with time spent facing in a  
818 different direction. Low directional drive results in lower speed (dashed arrow) and  
819 eventually peaking. Here memorised views are assumed to be facing upward and are  
820 thus recognised only when facing upward during peaking (small grey arrow) and not  
821 while facing downward during backward motion. Note that the second peek triggers a  
822 lower directional drive than the first (see also Fig. 1b).



1 **How do backward walking ants (*Cataglyphis velox*) cope with navigational**  
2 **uncertainty?**

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22 **ABSTRACT**

23 Current opinion in insect navigation assumes that animals need to align with the goal  
24 direction to recognise familiar views and approach it. Yet, ants sometimes drag  
25 heavy food items backward to the nest and it is still unclear to what extent they rely  
26 on visual memories while doing so. In this study displacement experiments and  
27 alterations of the visual scenery reveal that ants do indeed recognise and use the  
28 learnt visual scenery to guide their path towards the nest while walking backward. In  
29 addition, the results show that backward ants estimate their directional uncertainty by  
30 integrating multiple cues such as visual familiarity, the state of their path integrator  
31 and the time spent backward. A simple mechanical model based on repulsive and  
32 attractive visual memories captures the results and explains how visual navigation  
33 can be performed backwards.

34

35 **Keywords:** ants, backward movement, navigation, peeking, route following,  
36 uncertainty, view-based navigation

37

38 Total word count: 7779

39

## 40 INTRODUCTION

41

42 Central place foragers – such as desert ants – exhibit formidable navigational  
43 skills to find food and their way back home during numerous daily foraging trips  
44 (Collett, Graham, & Durier, 2003; Heinze, Narendra, & Cheung, 2018; Wehner,  
45 2003). These ground dwellers rely on a set of navigational strategies such as path  
46 integration (Wehner & Srinivasan, 2003; Wittlinger, Wehner, & Wolf, 2006) and visual  
47 scene navigation (Cheng, Narendra, Sommer, & Wehner, 2009; Zeil, 2012). Path  
48 integration (PI) is the capacity to return to a starting point by constantly updating the  
49 directional and distance information (based on celestial compass cues and a stride  
50 odometer) during an outbound trip whereas visual scene navigation provides  
51 directional information by comparing memorised and currently perceived views of  
52 terrestrial cues. The literature agrees that ants continuously integrate the directional  
53 dictates of these different strategies, rather than switching between them (Collett,  
54 2012; Hoinville & Wehner, 2018; Legge, Wystrach, Spetch, & Cheng, 2014; Reid,  
55 Narendra, Hemmi, & Zeil, 2011; Wystrach, Mangan, & Webb, 2015).

56 Current models of insect visual navigation capture well the behaviour of  
57 forward navigating ants (Baddeley, Graham, Husbands, & Philippides, 2012;  
58 Hoinville & Wehner, 2018; Wystrach, Beugnon, & Cheng, 2011; Wystrach, Cheng,  
59 Sosa, & Beugnon, 2011; Zeil, 2012) however, how ants navigate while dragging a  
60 heavy food item backward remains unclear (Ardin, Mangan, & Webb, 2016; Pfeffer &  
61 Wittlinger, 2016; Schwarz, Mangan, Zeil, Webb, & Wystrach, 2017). Despite their  
62 irregular backward foot strides the ants' PI system seems as accurate as during  
63 forward movement (Pfeffer, Wahl, & Wittlinger, 2016; Pfeffer & Wittlinger, 2016),  
64 however, guidance based on terrestrial visual cues seems disrupted (Schwarz et al.,  
65 2017). Indeed, evidence suggests that to recognise the familiar terrestrial scenery

66 ants need to align their body in the familiar forward direction (Narendra, Gourmaud, &  
67 Zeil, 2013; Wystrach, Cheng, et al., 2011; Zeil, 2012). This is probably why ants  
68 dragging a food item backward occasionally display a so-called 'peeking' behaviour:  
69 the ant stops pulling, drops its food item and turns around to look forward. If the  
70 scenery is familiar, the ant quickly returns to her food item and adjusts her backward  
71 path in the newly corrected homing direction. It seems clear that during these few  
72 moments facing forward in a familiar direction, ants recover and store the correct  
73 direction; and subsequently rely on celestial cues to maintain this new bearing when  
74 traveling backward (Schwarz et al., 2017). In this case navigation is discretised into  
75 different sources of information being used sequentially rather than simultaneously.  
76 Also, 'peeking' involves the decision to trigger a distinct and observable behaviour  
77 when navigational information is needed. This behaviour therefore provides a good  
78 opportunity to investigate how ants estimate their navigational uncertainty and as a  
79 corollary, which navigational information they have access to.

80 Here two experiments with backward walking ants were carried out to  
81 investigate the following questions: (1) Do ants still pay attention to visual terrestrial  
82 cues when walking backward? (2) Does this visual information enable ants to guide  
83 their backward path? (3) Which information is used by ants to estimate uncertainty  
84 and trigger a peeking behaviour?

85

## 86 **METHODS**

87

### 88 **Study animal and site**

89

90 The experiments were carried out with Spanish desert ants *Cataglyphis velox*  
91 on a field site with diverse grass and bush vegetation at the outskirts of Seville

92 (37°19'55"N 5°59'20"W) during June 2017 and 18. *Cataglyphis velox* show typical  
93 characteristics of a desert ant such as diurnality, thermophily and solitary foraging  
94 (Cerde, 2001). As in other ant species, navigation and orientation in *C. velox* is  
95 predominantly based on vision derived from terrestrial and celestial cues (Mangan &  
96 Webb, 2012; Wystrach et al., 2015).

97

## 98 **General methods**

99 Two experiments were conducted: Experiment 1 in 2017 and Experiment 2 in  
100 2018. Both set-ups shared the following methods. Ants were restricted to forage on a  
101 straight route (for specific dimensions see below) between their nests and a feeder.  
102 The routes were cleared from all vegetation that could interfere with ant observations  
103 and enclosed by thin white plastic planks (10 cm high) that were dug halfway into the  
104 ground. The slippery surface of the planks prevented ants foraging elsewhere while  
105 minimising the obstruction of surrounding views (Wystrach, Beugnon, & Cheng,  
106 2012). Ants could freely travel between nest and feeder, which was a ~ 15 × 15 × 15  
107 cm plastic bowl sunk into the ground that contained several kinds of sweet buttery  
108 biscuit crumbs. The walls of the bowl were covered with a thin layer of Fluon® and  
109 prevented ants from climbing. Ants that dropped into the feeder and picked up a  
110 crumb were marked individually with coloured acrylic or enamel paint (Tamiya™).  
111 During training, ants could leave the feeder via a small wooden ramp. Ants were  
112 considered trained and ready for testing once they had performed at least five  
113 foraging runs and were able to reach the feeder from the nest in a straight line  
114 (without colliding into any barriers). During tests (see below) the feeder ramp was  
115 removed to prevent other homing ants from interfering.

116

## 117 **Experiment 1**



118

119 Experiment 1 was conducted to assess the occurrence of peeking behaviour  
120 in backward walking ants across four different test conditions with one nest of  
121 *Catalgyphis velox* (summer 2017) located at the beginning of a straight 8.0 m × 1.8 m  
122 long foraging route. The length of the route was chosen to be long enough for ants to  
123 build up proper PI information but short enough to enable the ants to drag a crumb  
124 the whole distance without being too fatigued. Three thin (0.5 cm width), large  
125 wooden boards (2.4 m × 1.2 m) were connected (7.2 m × 1.2 m) and placed onto the  
126 foraging route (Fig. 1a, grey rectangles). These boards enhanced the tarsi grip of the  
127 ants and provided an even substrate that minimised potential interference with small  
128 grass haulms or pebbles during tests when ants dragged their food items backward.

129 During training, the individually marked foragers scuttled (forward) between  
130 the nest and feeder over the connected boards and familiarised themselves with the  
131 visual surroundings. After training, individual ants were subjected to one test  
132 conditions. All tests comprised of a forager that dragged a large biscuit crumb  
133 backward. For that, trained foragers with a small food item (~ 0.2 × 0.2 × 0.2 cm)  
134 were caught and transferred into a plastic vial. The food item was then carefully and  
135 manually removed and a larger biscuit piece (~ 2.0 × 0.5 × 0.2 cm) was offered to the  
136 ant instead. The biscuit provided was large enough to force the ants to drag it  
137 backward. After the ant locked mandibles onto the large biscuit, she was transferred  
138 to the appropriate release point. Four possible test conditions were carried out with  
139 either FV- (i.e., ants with their PI vector information, captured at the feeder) or ZV-  
140 ants (i.e., foragers without PI information, captured just before they enter the nest;  
141 Fig. 1a). To test whether ants pay attention to the visual scenery during backward  
142 motion, FV ants were released at the feeder either with the familiar scenery (FV,  
143 condition 1) or with altered visual surroundings (FVA, condition 2): by adding large

144 black plastic bags (~ 0.8 m × 0.6 m) on one side and a large dark tarp (0.9 m × 3.4  
145 m) on the other side of the route (Fig. 1a, right panel) to avoid potential obstructions.  
146 The objects were added a few seconds after the FVA-ant had started to home  
147 backward to ensure that the ant did not perceived any alteration before. To test the  
148 effect of route location, backward ants were released either at the beginning (ZV,  
149 condition 3) or at the middle of the route (ZVmid, condition 4).

150 It should be noted that upon release, the ants tested in Experiment 1 had the  
151 chance to glimpse forward while handling their large biscuit before engaging in  
152 backward movements.

153

#### 154 **Experiment 1: data and analysis**

155

156 For all tests, the distance between the release point and the location at which  
157 peeking behaviours occurred was noted with the help of a measuring tape placed on  
158 the side of the board that spanned from the feeder to the nest. Tests ended as soon  
159 as the backward walking ants reached the end of the wooden boards (i.e., ~0.5 m in  
160 front of the nest entrance) or abandoned her food item for more than one minute.

161 Individual ants were tested only once per test but were subjected to different test  
162 conditions with at least one un-interfered training trial between tests. The sequence  
163 of tests was evenly counter-balanced across individuals (e.g., FV-FVA-ZV-ZVmid;  
164 FVA-ZV-ZVmid-FV; ZV-ZVmid-FV-FVA etc.)

165 Comparison were made between FV- vs. FVA-ants and ZV- vs. ZVmid-ants  
166 (Fig. 1a). Given the large inter-individual variations, paired-data was applied and thus  
167 individuals that were not tested on both FV and FVA or ZV and ZVmid conditions  
168 were excluded from the analysis. We compared both the distance at which the first  
169 peeking behaviour occurred (1<sup>st</sup> peek distance) and the overall peek rate of

170 individuals (i.e., number of peek/distance walked) using Wilcoxon rank sum tests, a  
171 nonparametric statistic for paired data. Given that all ants walked rather straight  
172 toward the nest along the route, distance walked could be simply approximated by  
173 the beeline distance walked along the route. Most ants walked the full route (7.2 m)  
174 except obviously ants in the ZVmid condition and some foragers that abandoned  
175 their biscuit. For the comparison of peek rate, the 7.2 m long route on the boards was  
176 divided into half (Section 1: 0 - 3.4 m; Section 2: 3.4 - 7.2 m; Figure 1a). Thus, during  
177 ZVmid tests ants ran only Section 2. Comparisons between ZV- vs. ZVmid-ants were  
178 conducted to separate the effect of *distance walked* (i.e., ZVmid vs. ZV on Section 1)  
179 from the *actual location* along the route (i.e., ZVmid vs. ZV on Section 2). Bayesian  
180 statistics based on the Z values from these tests were applied to evaluate which of  
181 these alternative hypotheses explain peek rate best. We used  $f(Z)$ , that is,  
182 the y values on the standard normal distribution obtained from each of  
183 the Z scores, to estimate evidence in favour of each hypothesis. The ratio  
184 of the  $f(Z)$  measures of the two hypothesis gives an estimate of the 'evidence  
185 ratio'. While evidence ratios under 5 are weak, ratios over 10 are very strong (see  
186 Wystrach et al. 2014 for more details).

187         Backward paths were recorded by using GoPro HERO3+ cameras which  
188 were manually held approx. 0.6 m above the tested ant. Therefore, a quantification of  
189 the movement speed of the ants before and after peeking could be calculated. We  
190 measured the relative distance walked by the backward ants during the five seconds  
191 preceding the onset of the first peek (i.e., before the moment when the ants released  
192 the biscuit) and five seconds after the peek (i.e., after the ant resumed backward  
193 motion).

194

195 **Experiment 2**

196

197 Experiment 2 was conducted to test whether ants could guide their path  
198 backwards based on terrestrial cues. To do so, we recorded the paths of backward  
199 walking ants captured near their nest and released at four different release points  
200 (summer 2018). We used two different nests of *C. velox* ants. For each nest, a 5.0 m  
201 × 2.0 m straight foraging route was built with the nest entrance at one end and the  
202 feeder at the other end (Fig. 2a). As in Experiment 1, the route was enclosed by  
203 white plastic planks and ants were given a choice of biscuit crumbs inside the feeder  
204 to prompt foraging. However, here the ants scuttled back and forth directly on the  
205 natural ground during training. Once trained (see General methods), individual ants  
206 were captured on their way home 0.5 m before reaching their nest and subsequently  
207 released at one out of four possible locations (Fig. 2a):

208 Feeder (F): Ants were released 0.5 m after the feeder toward the nest direction.

209 Before nest (BeN): Ants were released on the route, 0.5 m before their nest.

210 Beyond nest (ByN): Ants were released 0.5 m beyond the nest in the feeder-nest  
211 direction.

212 Unfamiliar (U): Ants were released ~ 30.0 m away from the nest in a visually  
213 unfamiliar location.

214 For all tests, ants were captured in a plastic vial, offered a large biscuit crumb  
215 to incite backward walking and, once the ant had grabbed the cookie, released within  
216 a lampshade at the middle of a large wooden board (2.4 m × 1.2 m). Individual ants  
217 were tested only once per test, but could be subjected to different tests conditions,  
218 with at least one un-interfered training trial between tests. The wooden board was set  
219 in place just before and removed just after each test. The board was centred on the  
220 current release location with the long side along the feeder-nest direction (Fig. 2a) as  
221 it represents the expected homing direction. The board provided an even substrate

222 during tests and prevented ants to use potential familiar olfactory cues from the  
223 ground or the nest (BeN and ByN tests). A grid pattern (0.25 m × 0.25 m) drawn on  
224 the board enabled paths to be transcribed onto gridded paper. The lampshade (beige  
225 opaque fabric, 0.5 m diameter; 0.4 m height) surrounded the ant upon release and  
226 obstructed any familiar terrestrial views; the top of the lampshade was open providing  
227 the ant with celestial compass cues. Once the ant had pulled the large crumb  
228 backward for 0.1 m, the lampshade was lifted and the visual surrounding was  
229 revealed. This ensured that the ants could not utilise any familiar scenes before  
230 starting their backward path. This sets Experiment 2 apart from Experiment 1 where  
231 tested ants had the chance to glimpse forward before dragging the biscuit backward.

232

### 233 **Experiment 2: data and analysis.**

234

235 The backward paths and locations of peeking behaviour were noted. For each  
236 peek, the duration (i.e., the time the ant was not dragging the biscuit) was recorded.  
237 The actual trajectory of the path during forward motion was not recorded. Recording  
238 continued until the ants either reached the edge of the board or abandoned their  
239 large crumb for more than 1 min.

240 The recorded paths were digitised as (x, y) coordinates using GraphClick (Arizona  
241 Software). Path characteristics such as direction, meander and peek location were  
242 computed and analysed with Matlab™ (Mathworks, Matick, MA, USA). The meander  
243 index measures how much the path changes direction from segment to segment and  
244 was calculated as the mean angular deviation in direction between successive  
245 segments, each 0.3 m (Schwarz, Albert, Wystrach, & Cheng, 2011). Differences  
246 between test locations were determined using a generalised linear mixed effect  
247 model with repeated ants as random effect and Tukey's post hoc corrections. For

248 peek durations, a model for proportional (binomial) data was applied with 0 to 60 s  
249 (the duration at which recording was stopped) reported between 0 and 1.

250

## 251 **RESULTS**

252

### 253 **Experiment 1**

254

255 Experiment 1 was designed to investigate whether the occurrence of peeking  
256 behaviour in backward walking ants depends on factors such as: path integration,  
257 terrestrial cues, the location along the route or the distance walked.

258

#### 259 *FV vs. ZV: effect of path integration*

260 In both FV and ZV conditions, ants started to pull their biscuit toward the nest and  
261 mostly maintained that direction. However, the PI state had a visible effect on the  
262 occurrence of peeking behaviours: ZV-ants peeked earlier (first peek distance ZV-  
263 ants: mean  $\pm$  SD = 3.28  $\pm$  2.19 m; FV-ants: mean  $\pm$  SD = 5.90  $\pm$  1.93 m; Wilcoxon  
264 rank sum test:  $P < 0.001$ ,  $Z = -3.458$ ) and thrice as much (overall peek rate ZV-ants:  
265 mean  $\pm$  SD = 0.63  $\pm$  0.63 peek/m; FV-ants: mean  $\pm$  SD = 0.19  $\pm$  0.29 peek/m;  
266 Wilcoxon rank sum test:  $P < 0.001$ ,  $Z = -3.751$ ) than FV-ants (Fig. 1c, blue vs. green).  
267 Also, ZV-ants occasionally abandoned their large food item and did not resume  
268 backward movements (6 out of 33), whereas FV-ants never abandoned theirs (0 out  
269 of 32). A Fisher's exact test verified a significant difference ( $P = 0.032$ ). It seems  
270 clear that the lack (or conflicting) PI information decreases the ant's directional  
271 certainty.

272

#### 273 *FV vs. FVA: effect of altered visual familiarity*

274 To test the potential effect of the level of an altered visual scenery on backward  
275 walking ants, two conditions were conducted: (1) FV-ants homing backward on the  
276 unaltered, usual route, and (2) FVA-ants, homing backward on the same route but  
277 with altered visual surroundings (Fig. 1a). The alternation of the visual surrounding  
278 was effected only after the ants had started their journey backward, so that  
279 differences indicate that ants paid attention to terrestrial cues while walking  
280 backward. Results showed that FVA-ants peeked more often than FV-ants, however,  
281 this effect was weak, and reaches significance only in Section 2 (Wilcoxon rank sum  
282 test:  $P = 0.027$ ,  $Z = 3.751$ ) but not in Section 1 of the route (Wilcoxon rank sum test:  
283  $P = 0.688$ ,  $Z \sim 0$ ; Fig. 1d), due to a statistical floor effect. Indeed, a low rate of  
284 peeking in the first section of the route was expected, given that the path integration  
285 vector is longer and thus stronger at the beginning of the route home (Wystrach et  
286 al., 2015).

287 Second and most importantly, FVA-ants travelling in the altered visual  
288 environment displayed their first peek earlier along the route as compared to FV-ants  
289 on the familiar route (Wilcoxon rank sum test:  $P = 0.044$ ,  $Z = 2.016$ ; Fig. 1d).  
290 Together, this suggests that backward walking ants paid attention to terrestrial cues.

291 We also noted that, as for ZV-ants (see above), FVA-ants tested in the altered  
292 familiar condition abandoned their biscuits significantly more often than FV-ants  
293 (FVA: 6 out of 31 vs. FV: 0 out of 32; Fisher's exact test:  $P = 0.022$ ). Here again, it  
294 seems that the altered visual scenery decreases the directional certainty of backward  
295 walking ants.

296

297 *ZV vs. ZVmid: effect of location*

298 We investigated the potential effect of the location along the route by releasing  
299 zero vector ants either at the beginning (ZV) or in the middle of the familiar route

300 (ZVmid; Fig. 1a, c). Consequently, ZVmid-ants walked only Section 2, while ZV-ants  
301 moved along both sections. Ants displayed their first peek on average slightly earlier  
302 when released at the middle of the route (ZVmid) than when released at the  
303 beginning of the route (ZV; Wilcoxon rank sum test:  $P = 0.040$ ,  $Z = 2.062$ ; Fig. 1e).  
304 Also, the peek rate displayed by ZVmid-ants along Section 2 (the only section they  
305 walked) was higher than ZV-ants along Section 1 (Wilcoxon rank sum test:  $P =$   
306  $0.005$ ,  $Z = -2.814$ ) but similar to the peek rate displayed by these ZV-ants along  
307 Section 2 of the route (Wilcoxon rank sum test:  $P = 0.796$ ,  $Z = -0.2585$ ; Fig. 1e). A  
308 Bayesian evidence ratio (see Methods) was computed to estimate whether Section 1  
309 (i.e., different location, same distance walked) or Section 2 (i.e., same actual  
310 location, different distance walked) of ZV-ants' peek rate resembles most ZVmid-  
311 ants' peek rate. The obtained evidence ratio was 50.74 in favour of Section 2, which  
312 equals 'overwhelming evidence' for an effect on peek rate of the actual location along  
313 the route rather than the distance walked.

314

### 315 *Peeking and walking speed*

316 Interestingly, in all conditions, and for the vast majority of the individuals, ants walked  
317 backward on average twice as quickly after peeking than before peeking (Fig. 1b).  
318 This supports the idea that a peeking event increases the ant's directional certainty  
319 for some time.

320

## 321 **Experiment 2**

322

323 Experiment 2 was conducted to test whether ants could guide their path  
324 backwards using terrestrial cues. To do so, we recorded the paths of backward  
325 walking ants captured near their nest and released at four different release points.



326 Unlike as in Experiment 1 all tested ants in Experiment 2 were prevented from  
327 monitoring the visual surrounding before dragging their food item backward as a  
328 lampshade was blocking the whole panoramic view upon release (see Methods).  
329 Hence, any difference in the taken direction must result from visual information  
330 perceived while the ants were dragging their crumb backward – at least until they  
331 peeked for the first time.

332

### 333 *Ants can guide their backward path*

334 To see whether ant paths were directed in different direction across release  
335 locations, the Y-values – the end position along the feeder-to-nest line relative to the  
336 release point – were examined. The Y-values at the end of the foragers' path varied  
337 across released location (ANOVA:  $F = 21.96$ ,  $P < 0.001$ ; Fig. 2c). Ants released at  
338 the feeder were directed toward the nest and hence obtained the highest Y-values  
339 (Tukey's post-hoc test F vs. BeN, ByN and U:  $Z_s > 3.75$ ,  $P_s < 0.001$ ; Fig. 2c). Ants in  
340 unfamiliar tests (U) showed no directional preference along the Y-axis (Fig. 2c), as  
341 expected given the lack of familiar visual information at this location. Interestingly,  
342 ants from BeN and ByN conditions differed significantly in their final Y-values on the  
343 board (Tukey's post-hoc test BeN vs. ByN:  $Z = 3.47$ ,  $P = 0.003$ ). The medians of both  
344 of these groups are close to the actual nest location, showing that they used familiar  
345 visual cues to search at the nest (Fig. 2c). Differences between conditions could also  
346 be observed in path meander (ANOVA:  $F = 9.07$   $P < 0.001$ ). Ants from the feeder  
347 test showed straighter paths than ants from all other conditions (Tukey's post-hoc  
348 test F vs. BeN, ByN and U:  $Z_s > 3.68$   $P_s < 0.002$ ; Fig. 2c). No difference in meander  
349 among the remaining test conditions could be determined. Indeed, BeN-, ByN- and  
350 U-ants were expected to search on the board: BeN- and ByN-ants due to the  
351 proximity of the nest and U-ants due to the of the lack of familiar visual information.

352 Overall, these data show that ants could use familiar visual cues to adequately direct  
353 their backward paths.

354 Remarkably, analysis of the paths displayed purely backwards, before the first  
355 peek (or until the ant left the board if she did not peek), showed a similar pattern of  
356 results for both the Y-values reached (ANOVA:  $F = 11.37$   $P < 0.001$ ) and path  
357 meander (ANOVA:  $F = 3.52$   $P = 0.024$ ) (Fig. 2D). Despite purely backward  
358 movements, ants released at the feeder travelled significantly longer distances along  
359 the feeder-nest direction (Tukey's post-hoc test: F vs. BeN, ByN and U:  $Z_s > 3.29$   $P_s$   
360  $< 0.006$ ; Fig. 2d) and displayed straighter paths (Tukey's post-hoc F vs. ByN and U:  
361  $Z_s > 2.65$   $P_s < 0.03$ ; F vs. BeN:  $Z = 1.88$   $P = 0.235$ ; Fig. 2d) before peeking. The  
362 three other groups (BeN, ByN, U) were indeed expected to search on the board and  
363 to perform a similar level of path meander, as observed. Differences in the Y-values  
364 reached when released on each side of the nest did not reach significance using  
365 Tukey's post-hoc test (BeN vs. ByN:  $Z = 2.084$ ,  $P = 0.158$ ), however, the pattern of  
366 results followed what was expected if ants were using views to direct their path  
367 toward the nest. Ants released before (BeN) and beyond (ByN) their nest both moved  
368 on average toward the nest location, that is, in opposite direction from their release  
369 points; and ants released at the unfamiliar test site (U) showed less directed paths  
370 (Fig. 2d). The differences in paths characteristics is reflected if one considers the  
371 probability of obtaining the expected order of path endpoint across the four test  
372 conditions (Y-value:  $F > \text{BeN} > \text{U} > \text{ByN}$ ) is  $1/4! = 0.042$ .

373 Because nest-directed path sections were achieved before the ants triggered their  
374 first peek and the visual panorama was revealed to them only after they had started  
375 backward motion, the differences across locations show that ants can recognise and  
376 use the familiar visual cues to guide their path while moving backward and without  
377 the need of peeking.

378 Interestingly, several ants released at the feeder (4 out of 16 ants) displayed nest-  
379 directed backward paths across the whole recording board without performing a  
380 single peek and by keeping their body orientation away from the feeder-nest direction  
381 by at least 90° (bold paths in Fig. 2d).

382

### 383 *Peek duration and past information*

384 We also tested whether peek duration was influenced by the test condition and the  
385 number of previously displayed peeks (Fig. 2b). The feeder condition was excluded  
386 from this analysis as these ants were expected to move in a straight line and exit the  
387 board so that the actual peek number of a given ant may correlate with the location  
388 where the ant peeks. The three other groups (BeN, ByN, U) on the other hand, are  
389 expected to search on the board so any effect of the peek number is unlikely to be  
390 attributed to a specific location on the board. Interestingly, peek duration, which was  
391 recorded up to 60 s, was strongly influenced by the number of peeks previously  
392 displayed by the ant (GLM peek number:  $F = 17.09$ ,  $P < 0.001$ ; Fig. 2b) and not the  
393 actual test condition (GLM condition:  $F = 0.17$ ,  $P = 0.841$ ; Fig. 2b). The more peeks  
394 an ant had previously displayed the longer its current peeking lasts. This shows that  
395 the ant's peeking behaviour is also modulated by past information. Whether it is the  
396 time passed or the number of peek previously displayed cannot be disentangled  
397 here.

398

## 399 **DISCUSSION**

400

401 Ants dragging a heavy food item backward occasionally trigger a so-called  
402 'peeking behaviour' or 'peek': ants drop their food and turn around to look forward.  
403 Aligning their body in a familiar direction enables them to recognise the learnt visual

404 panorama and store this direction to follow it during their subsequent backward path  
405 (Schwarz et al., 2017). It is clear that ants gain directional information from learnt  
406 terrestrial cues when peeking forward. However, whether or not they use terrestrial  
407 cues while dragging their food item backward is less clear. Several of the current  
408 results demonstrate that ants are indeed able to do so, raising question about the  
409 underlying mechanisms.

410

### 411 **Ants still recognise terrestrial cues while walking backward**

412

413 Experiment 1 shows that the visual scenery experienced while walking backward  
414 influences the occurrence of peeking behaviour. First, zero vector (ZV) ants (i.e., ants  
415 captured at the nest and thus deprived of useful path integration (PI) information),  
416 displayed their first peek earlier when starting their backward journey halfway along  
417 the route than when starting at the beginning of the route (Fig. 1e). Second, full  
418 vector (FV) ants (i.e., captured at the feeder and thus having a PI vector pointing  
419 towards the nest) displayed their first peek earlier along the route if the surrounding  
420 scenery was artificially altered (FVA, Fig. 1d). This was true even though the scene  
421 was manipulated only after the ants had started dragging their biscuit backward  
422 indicating that ants paid attention to the familiar scenery while walking backward. It  
423 should be noted that this effect was weak (Fig. 1d), possibly because the alteration of  
424 the scene was not obvious enough (Schwarz et al., 2014).

425 In Experiment 2, ants could guide their trajectories based on terrestrial cues while  
426 walking backward. Ants were released on a board (ruling out the use of olfactory  
427 cues) and within a lampshade at different location relative to the nest (Fig. 2). The  
428 visual world was revealed to them only after they had started their backward journey.  
429 Nonetheless and despite the lack of PI homing vector, their paths were oriented in

430 the expected direction (i.e., the nest) resulting in differences between test conditions.  
431 Importantly, this was also true for the portion of path displayed before their first peek,  
432 that is, displayed purely backward (Fig. 2d).

433 In sum, ants can use learnt terrestrial visual cues while walking backward to guide  
434 their path as well as decide whether and when to peek forward. The next section  
435 discusses potential explanations.

436

### 437 **Mental rotation or combining attractive and repulsive views?**

438

439 How can ants recognise views backward? This is a puzzling question given that  
440 the assumption of current models of visual homing states that views must be  
441 retinotopically aligned to provide directional information (Ardin, Peng, Mangan,  
442 Lagogiannis, & Webb, 2016; Baddeley et al., 2012; Collett, Graham, & Collett, 2017;  
443 Le Möel & Wystrach, 2019; Möller, 2012; Murray et al., 2020; Narendra et al., 2013;  
444 Wystrach, Cheng, et al., 2011; Wystrach, Mangan, Philippides, & Graham, 2013;  
445 Zeil, Hofmann, & Chahl, 2003) although some other processes may be also at work  
446 (Wystrach et al., 2012). Recently, it has been suggested that ants may perform some  
447 sort of mental rotation to compare misaligned views (Ardin, Mangan, Wystrach, &  
448 Webb, 2015; Ardin et al., 2016), which may be achieved if views are encoded in the  
449 frequency domain (Stone et al., 2017). However, one can wonder why, if they can  
450 achieve mental rotation, would the ants peek at all? A capacity for mental rotation is  
451 hard to reconcile with the result of previous experiments where ants would not adjust  
452 their backward trajectory at all unless they peeked to align their body in the correct  
453 direction (Schwarz et al., 2017).

454 An alternative hypothesis would be that backward dragging ants may assess the  
455 maximum, rather the minimum of the rotational image difference function (Zeil et al.,

456 2003). However, this fails to explain why ants succeed to use terrestrial cues to guide  
457 their path backward on a two-way route but not on a one-way route (Schwarz et al.,  
458 2017). Also, to assess the maximum (as for the minimum (Wystrach et al., 2015)) for  
459 homing requires ants to scan the world to evaluate the relative mismatch across the  
460 different rotations; here however, the ants could maintain a straight backwards path  
461 without scanning (Fig. 2d).

462 Here we suggest instead a third hypothesis: ants may still need to align their body  
463 in the familiar direction to recognise memorised views but possess a memory bank of  
464 views learnt while facing in multiple directions and not only toward the nest – as  
465 suggested in a related manner concerning learning- and orientation walks (Zeil &  
466 Fleischmann, 2019). Notably, views learnt while facing in the anti-nest direction could  
467 be treated as repulsive when homing (Fig. 3a). The familiarities resulting from the  
468 comparison of the currently perceived view with both attractive (nest facing) and  
469 aversive (feeder facing) visual memories could simply be compared in a way  
470 somewhat analogous to an opponent process, as recently modelled in the light of the  
471 insect's neural circuitry (Le Möel & Wystrach, 2019). The signal resulting from this  
472 comparison informs the ant about whether to move toward or away from the currently  
473 faced direction, without the need for scanning (Le Möel & Wystrach, 2019). The only  
474 additional assumption needed for this model to explain backward guidance is that, if  
475 the ant is holding a large food item, 'moving away' means going backward because  
476 the ant cannot lift the cookie to turn its body. Fig. 3a illustrates the functionality of this  
477 simple model.

478 In the light of this model, we can wonder whether ants on the way home can also  
479 use the visual memories stored during their outbound trips (i.e., when they went from  
480 the nest to the feeder) as repulsive. This idea challenges the opinion that ants treat  
481 in- and outbound trip visually separately depending on the motivational state (Harris,

482 Hempel de Ibarra, Graham, & Collett, 2005; Wehner, Boyer, Loertscher, Sommer, &  
483 Menzi, 2006). Instead, ants may always recall both their memorised inbound and  
484 outbound facing views but treat them as repulsive or attractive depending on their  
485 current motivational state.

486

487 This attraction/repulsion model explains several observed phenomena of the  
488 current and past studies. (1) In a former experiment (Schwarz et al., 2017) backward  
489 ants were not able to correct their path at all while walking backward because, in this  
490 particular set-up, in- and out-bound routes were spatially separated (as a one-way  
491 circuit) so that no outbound views were available to potentially help out backward  
492 homing ants. (2) In the current Experiment 2, backward ants released at the feeder  
493 (F) carried on in the correct nest direction (Fig. 2d) because they recognised  
494 outbound views oriented toward the feeder, driving them away from (or opposite to)  
495 this direction (Fig. 3a). (3) Alteration of the visual surrounding would trigger earlier  
496 peeking behaviours because the familiarity of the feeder facing (outbound) views  
497 would be equally altered, disrupting the repulsive effect and thus reducing the overall  
498 directional drive (Fig. 1d). (4) Assuming that outbound views near the feeder are  
499 more familiar than in the middle of the route (ants perform learning walks at the  
500 feeder: (Nicholson, Judd, Cartwright, & Collett, 1999), the repulsive effect would be  
501 stronger for ants released at the feeder than in the middle of the route, yielding to a  
502 stronger directional drive and hence less peeking near the feeder (Fig. 1d). (5)  
503 Further, it was surprising in Experiment 2 that ants released close to the nest could  
504 direct their backward paths toward the nest (BeN, ByN; Figure 2c, d). Although this  
505 verifies that they recognised familiar views they nonetheless tended to peek often  
506 and even abandoned their cookie close to the nest (9 out of 28 in BeN- and 4 out of  
507 20 in ByN-test). This seems counter-intuitive, yet it can be explained in the light of the

508 integration between 'attractive and 'repulsive view'. During learning walks around the  
509 nest, ants appear to store indeed both nest and anti-nest oriented views (Jayatilaka,  
510 Murray, Narendra, & Zeil, 2018). Even if these nest views may be all extremely  
511 familiar, the integration of attractive (nest-oriented) and repulsive (anti-nest oriented)  
512 views would result in a low overall directional drive, and thus lead to high peek rates  
513 (and a high probability for abandoning the crumb) but nonetheless guide the ants  
514 toward the nest. This very same idea explained the recent puzzling observation that  
515 ants behaved similarly at the nest and in unfamiliar settings, but not on the foraging  
516 route (Murray et al., 2020). (6) Finally, two recent studies revealed the importance of  
517 outbound trips for homing ants. Ants with outbound views during training were more  
518 efficient in homing than ants with only inbound views during training (Freas & Cheng,  
519 2018; Freas & Spetch, 2019). But whether homing ants actually used their outbound  
520 view as suggested here, or simply learnt homing views by turning back during their  
521 outbound trips remains to be seen.

522

523 **Ants combine multiple cues to estimate directional uncertainty and trigger**  
524 **peeks.**

525

526 It is known that ants combine the directional dictates of the current visual  
527 familiarity with their PI in a weighted fashion (Wehner, Hoinville, Cruse, & Cheng,  
528 2018). Notably, the direction indicated by the current view is more weighted as the  
529 current view is familiar (Legge et al., 2014) and the direction indicated by the PI is  
530 more weighted as the PI vector length increases (Wystrach et al., 2015). Backward  
531 walking ants appear to weight these cues in the same fashion. Fig. 3b shows how  
532 such an integration of cues captures the peek rate observed across our conditions  
533 and distance walked along the route. Notably, this explains why ZV-ants peeked



534 earlier and more often than FV-ants (Fig. 1b), as observed in North African  
535 *Cataglyphis fortis* ants (Pfeffer & Wittlinger, 2016) and why peek rate increases as  
536 the distance walked along the route increases (Fig. 1d, e), as observed in *Myrmecia*  
537 *croslandi* ants (see Fig. S2 in Schwarz et al. 2017).

538 Interestingly, such an estimate of directional certainty seems not only to influence  
539 the occurrence of peeking but also whether the peeking ants decided to return to  
540 their biscuit or abandon it. FV-ants in familiar visual surroundings and therefore with  
541 the highest directional certainty, dragged their biscuit all along the 7.20 m route  
542 without exception (32 out of 32). In contrast, some ants abandoned their biscuits in  
543 both FVA (6 out of 31) and ZV (6 out of 33) conditions.

544 Finally, it is worth mentioning that ants clearly increased the speed of their  
545 backward motion after peeking (Fig. 1c). The increase in speed is likely not only a  
546 mere consequence of a short recovery from the dragging activity but also a gain in  
547 navigational certainty as this sudden speed increase is also apparent when displaced  
548 foragers reach their familiar route and recognise the familiar scenery (pers. observ.).  
549 Furthermore, a decrease in speed when ants run off their PI has been described  
550 (Buehlmann, Fernandes, & Graham, 2018). Hence it seems that the speed of  
551 movement reflects the strength of the directional drive and therefore directional  
552 certainty. If peeking behaviour seems to increase directional certainty, the later seem  
553 to decrease with time spent backwards (Fig. 3c), which also make functional sense.

554

#### 555 **Ants gather information about the time spent backward.**

556

557 Recently, it has been shown that directional information based on terrestrial cues is  
558 obtained when ants face forward during peeks and must therefore be stored in a  
559 short-term memory while the ant is subsequently walking backward (Schwarz et al.,

2017). Our results suggest that short-term memory also influences the ants' navigational certainty. The time spent peeking forward varied (as already noted by Pfeffer and Wittlinger (2016)) with some ants 'peeking' for less than a second while other spent more than 60s (after which recording was stopped) without returning to their cookie. Interestingly, the more an ant had peeked before during a test run, the longer her current peek lasted (Fig. 2b). This shows that ants somehow gather information across time to adjust their current certainty: either information about the overall time spent backward or information about the number of peek previously displayed (see also Fig. 3c).

Former experiments have already shown that the behaviour of ants can be modulated by recent experience in the order of seconds to minutes – a form of hysteresis (Graham & Mangan, 2015). For instance, ants display a so-called backtracking behaviour only if they have recently perceived the nest surrounding (Wystrach, Schwarz, Baniël, & Cheng, 2013). Also, homing ants display higher meander in their paths when recapitulating a well-known route for the second time in a row (Collett, 2014; Wystrach, Schwarz, Graham, & Cheng, 2019). Finally, ants can remember the compass direction of a wind gust after being blown (Wystrach & Schwarz, 2013). In the present case, ants seem to also build up information about the recent past. Even though it makes functional sense it remains to be seen what neural or physiological mechanisms underlie this phenomenon.

580

## 581 **Conclusion**

582 This study confirmed that ants walking backward are not just paying attention to  
583 celestial cues but combine multiple information from their PI, the recognition of  
584 terrestrial cues and temporal information such as the time they spent backward. All  
585 this information seems to be integrated in an overall directional drive. This drive,

586 which reflects the current directional certainty, seems to (1) guide the ants backward  
587 path, (2) triggers peeking behaviour if too low and (3) finally dictates whether or not  
588 to return to their food item during peeks. Importantly, this study shows that ants uses  
589 familiar terrestrial cues while navigating backward. In addition to the attractive  
590 memories facing toward the nest, the hypothesis that homing ants use a collection of  
591 repulsive visual memories facing away from the nest and possibly stored during their  
592 outbound trip was put forward (Le Möel & Wystrach, 2019). In the light of this  
593 hypothesis, visual navigation forward or backward can then simply be achieved by  
594 using the relative familiarity between both sets of opposite valence memories. As  
595 often with research on insect navigation, remarkably flexible behaviours incite  
596 researchers to endorse the simplest explanations (Wystrach & Graham, 2012).

597

#### 598 **Author contributions**

599 Conceptualisation: S.S., A.W.; Methodology: S.S., A.W.; Formal analysis: S.S., A. W.  
600 Investigation: S.S., L.C., E.G., A.W.; Writing - original draft: S.S.; Writing - review &  
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602

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611 **References**

612

613 Ardin, P., Mangan, M., Wystrach, A., & Webb, B. (2015). How variation in head pitch  
614 could affect image matching algorithms for ant navigation. *Journal of*  
615 *Comparative Physiology A*, 201, 585–597.

616 Ardin, P., Peng, F., Mangan, M., Lagogiannis, K., & Webb, B. (2016). Using an insect  
617 mushroom body circuit to encode route memory in complex natural  
618 environments. *PLoS Computational Biology*, 12, e1004683.

619 Ardin, P. B., Mangan, M., & Webb, B. (2016). Ant homing ability is not diminished  
620 when traveling backwards. *Frontiers in Behavioral Neuroscience*, 10:69.

621 Baddeley, B., Graham, P., Husbands, P., & Philippides, A. (2012). A Model of Ant  
622 Route Navigation Driven by Scene Familiarity. *PLoS Computational Biology*,  
623 8, 1002336.

624 Buehlmann, C., Fernandes, A., & Graham, P. (2018). The interaction of path  
625 integration and terrestrial visual cues in navigating desert ants: what can we  
626 learn from path characteristics? *The Journal of Experimental Biology*, 221.

627 Cerda, X. (2001). Behavioural and physiological traits to thermal stress tolerance in  
628 two Spanish desert ants. *Etologia*, 9, 15-27.

629 Cheng, K., Narendra, A., Sommer, S., & Wehner, R. (2009). Traveling in clutter:  
630 navigation in the Central Australian desert ant *Melophorus bagoti*. *Behavioural*  
631 *Processes*, 80, 261-268.

632 Collett, M. (2012). How navigational guidance systems are combined in a desert ant.  
633 *Current Biology*, 22, 927-932.

634 Collett, M. (2014). A desert ant's memory of recent visual experience and the control  
635 of route guidance. *Proceedings of the Royal Society B: Biological Sciences*,  
636 281, 20140634.

637 Collett, M., Graham, P., & Collett, T. S. (2017). Insect navigation: what backward  
638 walking reveals about the control of movement. *27*, 141-143.

639 Collett, T., Graham, P., & Durier, V. (2003). Route learning by insects. *Current*  
640 *Opinion in Neurobiology*, *13*, 718-725.

641 Freas, C. A., & Cheng, K. (2018). Landmark learning, cue conflict, and outbound  
642 view sequence in navigating desert ants. *Journal of Experimental Psychology:*  
643 *Animal Learning and Cognition*, *44*, 409-421.

644 Freas, C. A., & Spetch, M. L. (2019). Terrestrial cue learning and retention during the  
645 outbound and inbound foraging trip in the desert ant, *Cataglyphis velox*.  
646 *Journal of Comparative Physiology A*, *205*, 177-189.

647 Graham, P., & Mangan, M. (2015). Insect navigation: do ants live in the now? *The*  
648 *Journal of Experimental Biology*, *218*, 819-823.

649 Harris, R. A., Hempel de Ibarra, N., Graham, P., & Collett, T. S. (2005). Priming of  
650 visual route memories. *Nature*, *438*, 302.

651 Heinze, S., Narendra, A., & Cheung, A. (2018). Principles of insect path integration.  
652 *Current Biology*, *28*, 1023-1058.

653 Hoinville, T., & Wehner, R. (2018). Optimal multiguide integration in insect  
654 navigation. *Proceedings of the National Academy of Sciences of the United*  
655 *States of America*, *115*(11), 2824-2829.

656 Jayatilaka, P., Murray, T., Narendra, A., & Zeil, J. (2018). The choreography of  
657 learning walks in the Australian jack jumper ant *Myrmecia croslandi*. *The*  
658 *Journal of Experimental Biology*, *221*, 185306.

659 Le Möel, F., & Wystrach, A. (2019). Opponent processes in visual memories: a  
660 model of attraction and repulsion in navigating insects' mushroom bodies.  
661 *bioRxiv*. doi:10.1101/2019.12.16.877449

662 Legge, E., Wystrach, A., Spetch, M., & Cheng, K. (2014). Combining sky and earth:  
663 desert ants (*Melophorus bagoti*) show weighted integration of celestial and  
664 terrestrial cues. *The Journal of Experimental Biology*, 217, 4159-4166.

665 Mangan, M., & Webb, B. (2012). Spontaneous formation of multiple routes in  
666 individual desert ants (*Cataglyphis velox*). *Behavioral Ecology*, 23, 944-954.

667 Möller, R. (2012). A model of ant navigation based on visual prediction. *Journal of*  
668 *Theoretical Biology*, 305, 118-130.

669 Murray, T., Zoltán, K., Dahmen, H., Le Möel, F., Wystrach, A., & Zeil, J. (2020). The  
670 role of attractive and repellent scene memories in ant homing (*Myrmecia*  
671 *croslandi*). *The Journal of Experimental Biology*, 223, jeb210021.

672 Narendra, A., Gourmaud, S., & Zeil, J. (2013). Mapping the navigational knowledge  
673 of individually foraging ants, *Myrmecia croslandi*. *Proceedings of the Royal*  
674 *Society B: Biological Sciences*, 280, 20130683.

675 Nicholson, D. J., Judd, S. P. D., Cartwright, B. A., & Collett, T. S. (1999). Learning  
676 walks and landmark guidance in wood ants (*Formica rufa*). *The Journal of*  
677 *Experimental Biology*, 202, 1831-1838.

678 Pfeffer, S., Wahl, V., & Wittlinger, M. (2016). How to find home backwards?  
679 Locomotion and inter-leg coordination during rearward walking of *Cataglyphis*  
680 *fortis* desert ants. *The Journal of Experimental Biology*, 219, 2110-2118.

681 Pfeffer, S., & Wittlinger, M. (2016). How to find home backwards? Navigation during  
682 rearward homing of *Cataglyphis fortis* desert ants. *The Journal of*  
683 *Experimental Biology*, 219, 2119-2126.

684 Reid, S. F., Narendra, A., Hemmi, J. M., & Zeil, J. (2011). Polarised skylight and the  
685 landmark panorama provide night-active bull ants with compass information  
686 during route following. *The Journal of Experimental Biology*, 214, 363-370.

687 Schwarz, S., Albert, L., Wystrach, A., & Cheng, K. (2011). Ocelli contribute to the  
688 encoding of celestial compass information in the Australian desert ant  
689 *Melophorus bagoti*. *The Journal of Experimental Biology*, 214, 901-906.

690 Schwarz, S., Julle-Daniere, E., Morin, L., Schultheiss, P., Wystrach, A., Ives, J., &  
691 Cheng, K. (2014). Desert ants (*Melophorus bagoti*) navigating with robustness  
692 to distortions of the natural panorama. *Insectes Sociaux*, 61, 371-383.

693 Schwarz, S., Mangan, M., Zeil, J., Webb, B., & Wystrach, A. (2017). How Ants Use  
694 Vision When Homing Backward. *Current Biology*, 27, 401-407.

695 Stone, T., Webb, B., Adden, A., Weddig, N., Honkanen, A., Templin, R., . . . Heinze,  
696 S. (2017). An anatomically constrained model for path integration in the bee  
697 brain. *Current Biology*, 27, 3069-3085.e3011.

698 Wehner, R. (2003). Desert ant navigation: how miniature brains solve complex tasks.  
699 *Journal of Comparative Physiology A*, 189, 579-588.

700 Wehner, R., Boyer, M., Loertscher, F., Sommer, S., & Menzi, U. (2006). Ant  
701 navigation: one-way routes rather than maps. *Current Biology*, 16, 75-79.

702 Wehner, R., Hoinville, T., Cruse, H., & Cheng, K. (2018). Steering intermediate  
703 courses: desert ants combine information from various navigational routines.  
704 *Journal of Comparative Physiology A*, 202, 459–472.

705 Wehner, R., & Srinivasan, M. V. (2003). Path integration in insects. In K. K. Jeffrey  
706 (Ed.), *The neurobiology of spatial behaviour* (pp. 9-30). Oxford: Oxford  
707 University Press.

708 Wittlinger, M., Wehner, R., & Wolf, H. (2006). The ant odometer: stepping on stilts  
709 and stumps. *Science*, 312, 1965-1966.

710 Wystrach, A., Beugnon, G., & Cheng, K. (2011). Landmarks or panoramas: what do  
711 navigating ants attend to for guidance? *Frontiers in Zoology*, 8, 21.

712 Wystrach, A., Beugnon, G., & Cheng, K. (2012). Ants might use different view-  
713 matching strategies on and off the route. *The Journal of Experimental Biology*,  
714 215, 44-55.

715 Wystrach, A., Cheng, K., Sosa, S., & Beugnon, G. (2011). Geometry, features, and  
716 panoramic views: Ants in rectangular arenas. *Journal of Experimental*  
717 *Psychology: Animal Behavior Processes*, 37, 420-435.

718 Wystrach, A., & Graham, P. (2012). What can we learn from studies of insect  
719 navigation? *Animal Behaviour*, 84, 13-20.

720 Wystrach, A., Mangan, M., Philippides, A., & Graham, P. (2013). Snapshots in ants?  
721 New interpretations of paradigmatic experiments. *The Journal of Experimental*  
722 *Biology*, 216, 1766-1770.

723 Wystrach, A., Mangan, M., & Webb, B. (2015). Optimal cue integration in ants.  
724 *Proceedings of the Royal Society B: Biological Sciences*, 282, 20151484.

725 Wystrach, A., & Schwarz, S. (2013). Ants use a predictive mechanism to compensate  
726 for passive displacements by wind. *Current Biology*, 23, 1083-1085.

727 Wystrach, A., Schwarz, S., Baniel, A., & Cheng, K. (2013). Backtracking behaviour in  
728 lost ants: an additional strategy in their navigational toolkit. *Proceedings of the*  
729 *Royal Society B: Biological Sciences*, 280, 20131677.

730 Wystrach, A., Schwarz, S., Graham, P., & Cheng, K. (2019). Running paths to  
731 nowhere: repetition of routes shows how navigating ants modulate online the  
732 weights accorded to cues. *Animal Cognition*, 22, 213–222.

733 Wystrach, A., Schwarz, S., Schultheiss, P., Baniel, A., & Cheng, K. (2014). Multiple  
734 sources of celestial compass information in the Central Australian desert ant  
735 *Melophorus bagoti*. *Journal of Comparative Physiology A*, 200, 591-601.

736 Zeil, J. (2012). Visual homing: an insect perspective. *Current Opinion in*  
737 *Neurobiology*, 22, 285–293.



738 Zeil, J., & Fleischmann, P. N. (2019). The learning walks of ants (Hymenoptera:  
739 Formicidae). *Myrmecological News*, 29, 93-110.

740 Zeil, J., Hofmann, M. I., & Chahl, J. S. (2003). Catchment areas of panoramic  
741 snapshots in outdoor scenes. *Journal of the Optical Society of America A*, 20,  
742 450-469.

743

744

745 **Figure 1.** Dynamics of peeking behaviours in terms of path integration and visual  
746 familiarity (Experiment 1). (a) Schematics of experimental set-up with training and  
747 test conditions. During training ants foraged between nest and feeder (~ 8.0 m) on  
748 three thin wooden boards serving as an even substrate (grey rectangles). The route  
749 was divided into two sections corresponding to the first and second half of the route.  
750 For tests, trained ants were either captured at the feeder (full-vector ants, FV) or just  
751 before entering the nest after foraging (zero-vector ants, ZV; open crosses) and  
752 released at the feeder (as FV- FVA or ZV-ants) or on the middle of the route (ZVmid-  
753 ants; black crosses). For FVA (altered familiarity), the familiarity of the route was  
754 manipulated by adding large black visual objects (black blobs) on one side and a  
755 dark tarp (black vertical bar) on the other side of the route. Dashed lines depict  
756 example paths of the ants. (b) Change in speed after the first peek. Each dot shows  
757 the relative change in speed (5s after/5s before) the first peek for each ant. Dotted  
758 line at 1 indicates no change in speed. (c) Cumulated number of peeks displayed  
759 against the distance walked along the route (mean  $\pm$  SE across individuals). (d)  
760 Overall peek rate (left) and distance of the first peek (right) for both FV-ant and FVA-  
761 ants. 7.3 m indicates the end of the route. (e) Overall peek rate (left) and distance of  
762 the first peek (right) for both ZV-ant conditions. 3.8 m indicates the end of the route  
763 (ZV-ant paths were truncated at 3.8 m to match the maximum homing distance of  
764 ZVmid-ants). Grey lines (d, e) represent individually tested ants across conditions.  
765 See main text for statistical details.

766

767 **Figure 2.** Backward path characteristics and peeking behaviour at different release  
768 points (Experiment 2). (a) Schematics of experimental set-up with training and test  
769 conditions. During training ants foraged between nest and feeder (~ 5.0 m). Trained  
770 ants with a remaining homing vector of 0.5 m (open cross depicts capture point) were

771 tested backward at different release points: feeder (F), before nest (BeN), beyond  
772 nest (ByN) and unfamiliar (U) test site. Ants were released (black crosses) on the  
773 middle of a thin wooden board (grey) to rule out the use of olfactory cues. Boards  
774 had a 0.25 m × 0.25 m squared pattern to enable path recording. Dashed lines depict  
775 example paths of ants during training. (b) Duration of peek (the time the ant spent  
776 away from the cookie) as a function of peek number for each individual. Max.  
777 threshold of peek duration was set to 60s and 'Feeder condition' was removed from  
778 this analysis as peek number correlates with position along the board. (c) Complete  
779 recorded backward paths of ants across conditions. Paths ended either because ants  
780 left the board or abandoned their cookie (peek duration > 60s). Boxplots show  
781 distance reached at the end of the path along the feeder nest axis (Y axis) and  
782 meander of the paths across individuals. Differences in top letters (a, b, c) indicate  
783 significant differences between groups (alpha = 0.05). Black dotted lines depict  
784 release point, black arrows remaining vector length (0.5 m) and open stars actual  
785 nest position. (d) As in (c), except that paths were truncated at the first peek or when  
786 exiting the board. Hence all navigational information gathered by ants was obtained  
787 while walking backward. Thick paths (left panel) emphasise four ants in the feeder  
788 conditions that displayed nest-directed backward paths without facing the nest  
789 direction. Open circles represent positions of 1<sup>st</sup> peeks. For statistical details see text.  
790

791 **Figure 3.** Models on how ants may combine navigational information. The longer the  
792 length of the drawn vectors the higher the directional drive. Dots represent cases with  
793 no directional drive and stars nest locations. (a) Illustration of the 'repulsive view  
794 hypothesis'. Overall directional drive results from the integration of attractive homing  
795 views (green arrows) minus repulsive outbound views (red arrows). Left scheme  
796 represents theoretical positions and orientations of memorised views. The ant

797 recognises only views that are aligned with its current body orientation (here, always  
798 facing downward). The larger the distance from the current location to the closest  
799 aligned view, the lower the familiarity and directional drive. This principle provides  
800 appropriate guidance toward the nest. Right scheme shows examples of integration  
801 for different locations (grey dashed arrows) with the ant always facing downward. For  
802 instance, when facing downward at the BeN location, the closest aligned view is  
803 repulsive (bold red arrow on right left scheme). Given that the neighbouring green  
804 arrow is not aligned, the closest aligned attractive view is further away, beyond the  
805 nest (bold green arrow on left scheme). Overall, the ants at this position (still facing  
806 downward) will be more repulsed than attracted by the current facing direction and  
807 thus walk backward toward the nest. In contrast, when beyond the nest ByN (still  
808 facing downward), the attractive views will match better than the repulsive views and  
809 the ants will thus turn around and walk backward toward the nest. Whatever the  
810 position and orientation of the ants around the nest, the agent will be drawn towards  
811 the nest (b) Directional drive across test conditions of Experiment 1 in Section 1 and  
812 2. Ants are tested in FV (full-vector), FVA (full-vector altered familiarity) and ZV (zero-  
813 vector) conditions. Overall directional drive (dark blue vectors) results from the  
814 integration of path integration (cyan vectors, the longer the path integration vector the  
815 stronger its directional drive) and learnt view (yellow vectors, the more familiar the  
816 view the stronger its directional drive). (c) Directional drive resulting from the  
817 recognition of a learnt view (yellow vectors) decrease with time spent facing in a  
818 different direction. Low directional drive results in lower speed (dashed arrow) and  
819 eventually peeking. Here memorised views are assumed to be facing upward and are  
820 thus recognised only when facing upward during peeking (small grey arrow) and not  
821 while facing downward during backward motion. Note that the second peek triggers a  
822 lower directional drive than the first (see also Fig. 1b).



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Figure 1

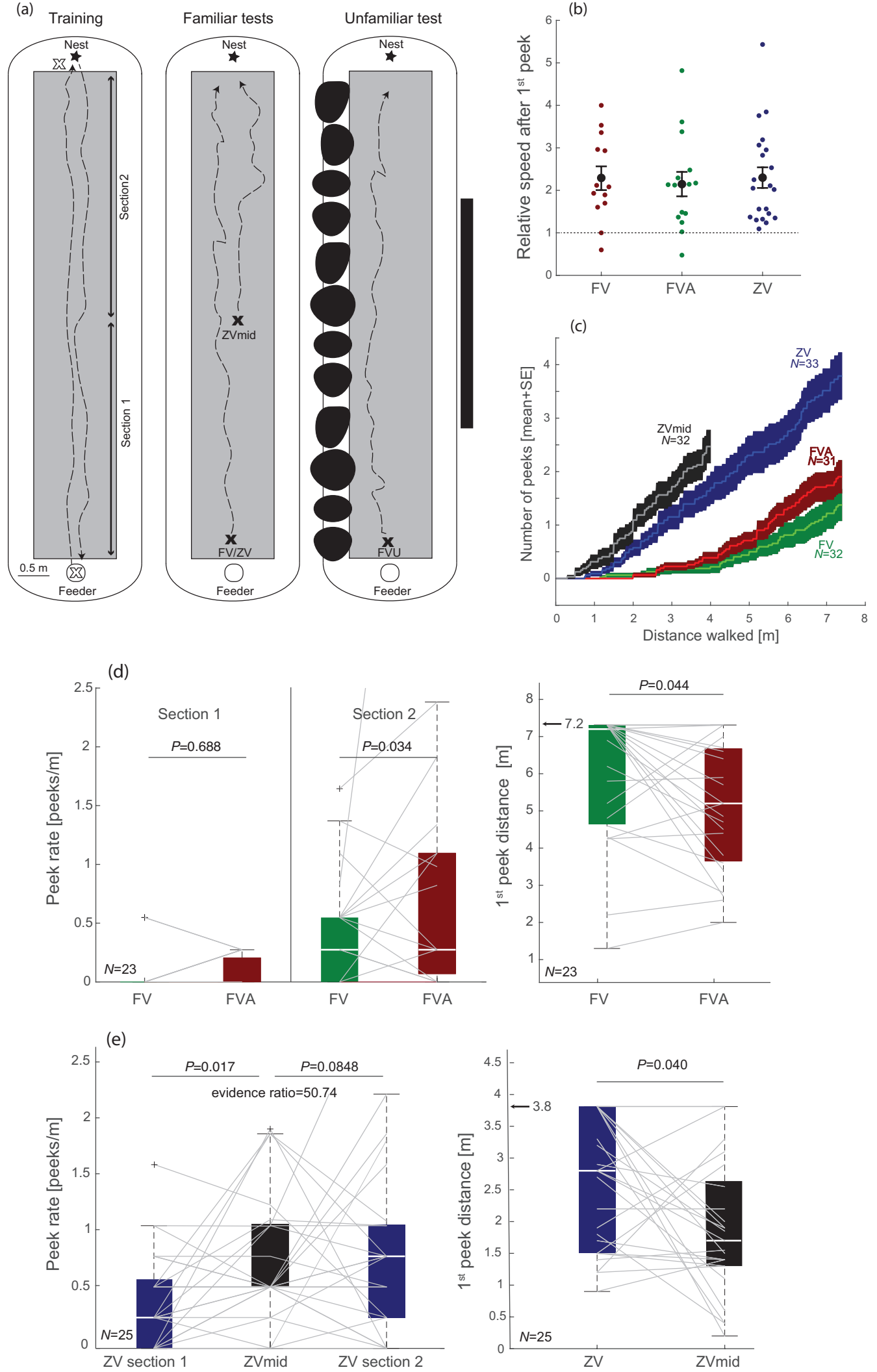
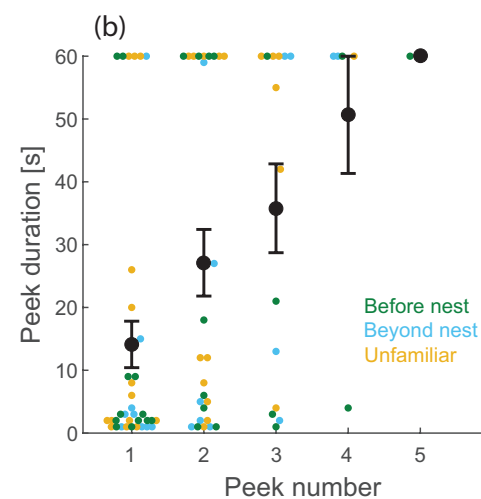
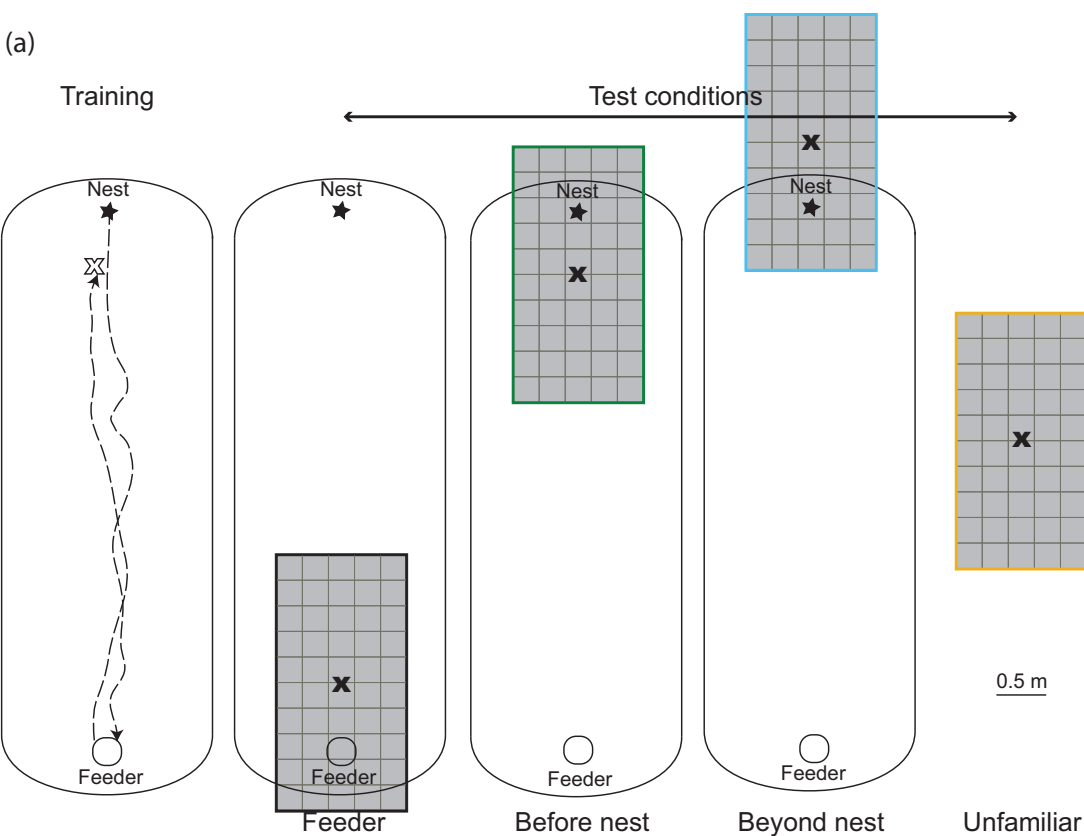
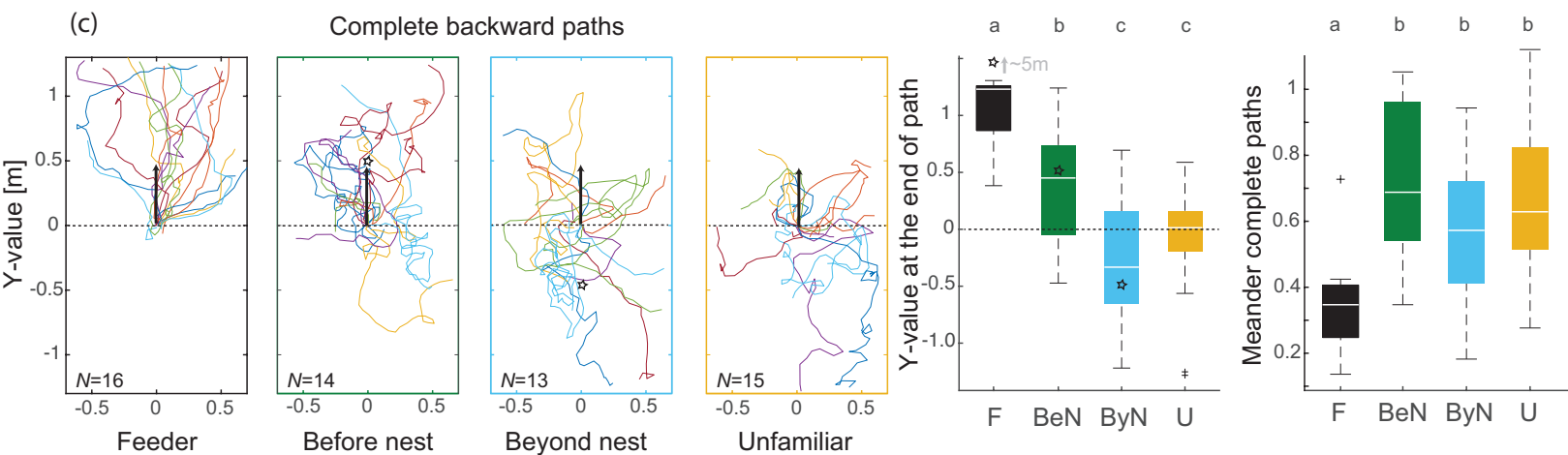


Figure 2

(a)



(c)



(d)

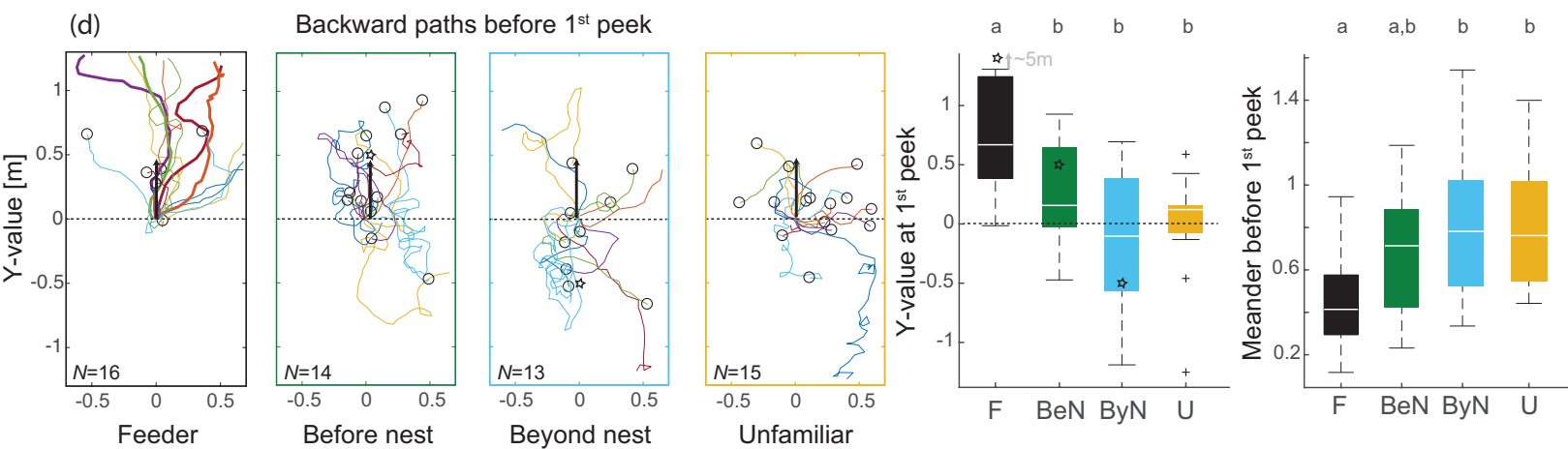
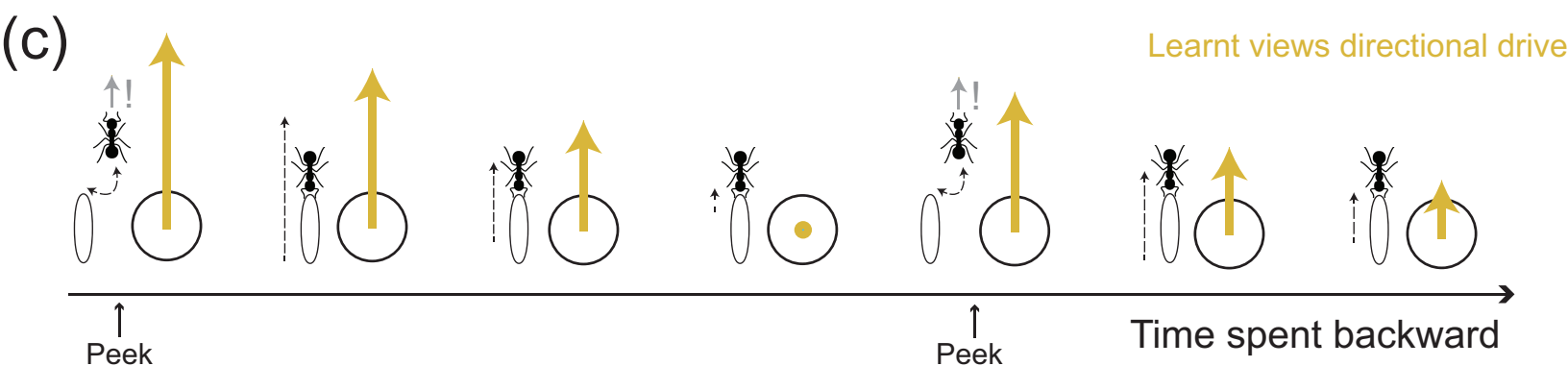
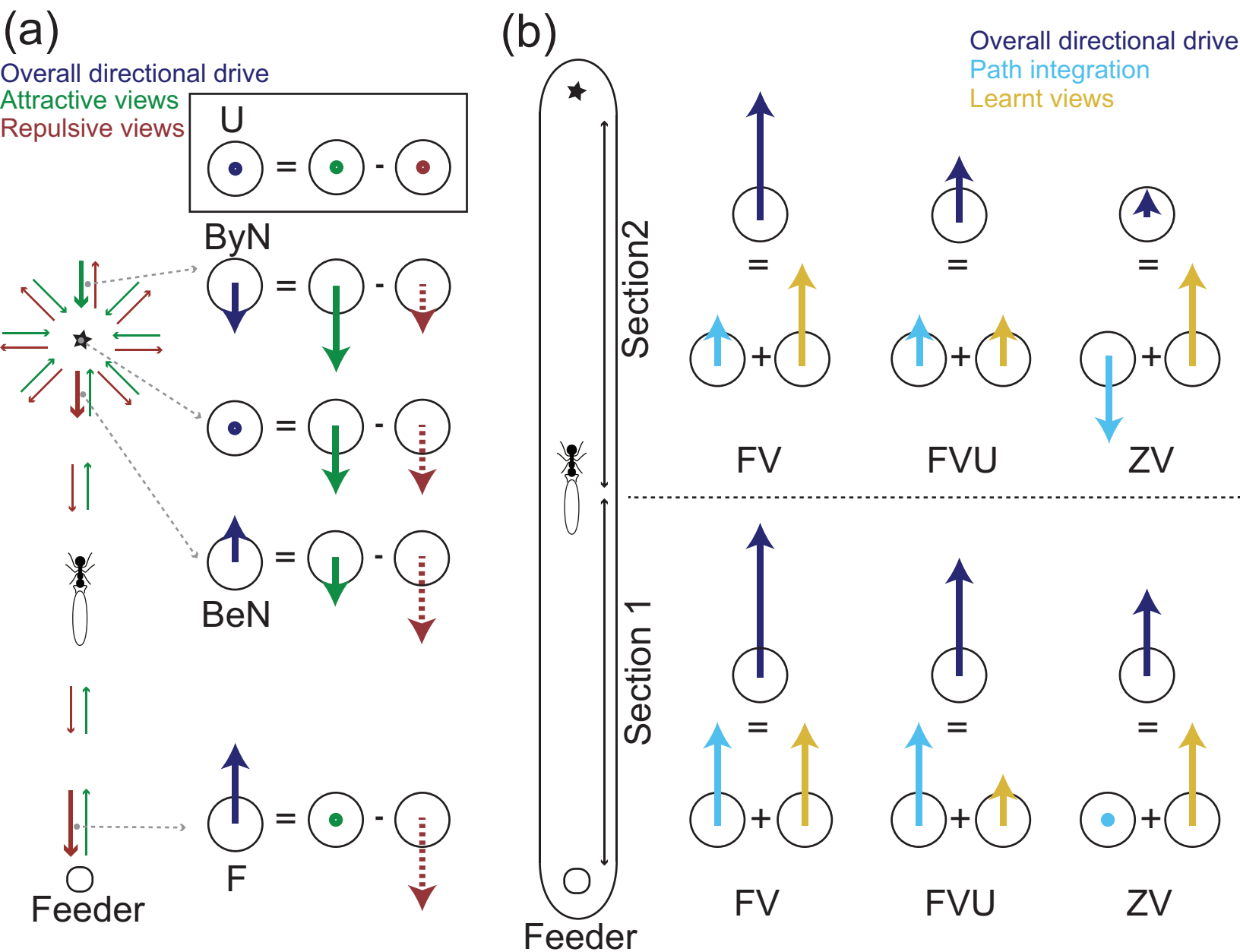




Figure 3



## **Ethical note**

Iberian *Cataglyphis velox* ants were used in the experiments. Ants were trained to forage at an artificial feeder. Once trained they were caught using a tube, marked with nonlethal paint and subjected to a test condition with no obvious signs of distress. No ants were killed during the experiment. As ants were already in the process of foraging, disturbance to their behaviour was minimal. As the work only involved invertebrates no licences were required